VOLUME 103 NUMBER 2

THE
BOTANICAL GAZETTE

December 1941

NUTRITION OF THE MYXOMYCETES. II. RELATIONS BETWEEN PLASMODIA, BACTERIA, AND SUBSTRATE IN TWO-MEMBERED CULTURE[1]

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(WITH ONE FIGURE)

Introduction

Under natural conditions, the myxomycetes are constantly found associated with microörganisms of many kinds, including bacteria, yeasts, molds, and protozoa. This association prevails not only during the vegetative stages of the myxomycetes but in the fruit as well, for sporangia have been found to contain the spores of bacteria and fungi and the cysts of protozoa, all extruded from the plasmodium during its fructification (**22**). Although this continual association with microörganisms has been noticed by many (**19**), the amount of sound correlative work on such relations is relatively slight. It has been mentioned previously (**5**) that factors other than the quantity of bacterial food operate in mixed cultures, and the present paper is an attempt to elucidate these factors.

Literature review

The reports of other workers on the relationships between myxomycete plasmodia and bacteria have been reviewed previously (**5**). It is established that plasmodia and myxamebae can ingest and digest a number of different microörganisms, but relatively few detailed studies have been made on the general types most readily ingested, the conditions governing ingestion, and the other factors concerned.

In considering the relations in two-membered cultures, two statements previ-

[1] Contribution from the Laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University, no. 188.

ously discussed (**5**) may be at once dismissed: CHRZĄSZCZ's (**2**) claim that the microörganisms and the myxomycetes are in competition for the supplied medium, and HENNEBERG's (**8**) conclusion that the myxomycete feeds mainly on the metabolic products of the microörganisms rather than on the microbes themselves.

SKUPIENSKI (**26**) has maintained that a symbiosis exists between each species of myxomycete and a particular species of bacterium, chiefly because plasmodia were often found associated each with a single species. From reports by other workers, and from the evidence presented here, it would not seem that a constant association in gross culture or in nature is alone a reliable criterion of symbiosis.

A further consideration of the relations between myxomycetes and bacteria apparently shows no correlation between the morphological types of the bacteria and the ability of plasmodia to assimilate them. WATANABE (**29**) found that although the five most suitable bacteria in his graded list were Gram positive, they agreed in no other respect; others found Gram-negative bacteria to be equally well assimilated (**28, 14**; cf. **5**). WATANABE's conclusions were based on the chemotaxis of plasmodia in association with the bacteria on nonnutrient agar; the suitability of the bacteria as food was judged by the degree to which the plasmodia followed and fed on the streak microörganisms; but, as shown later, this method cannot be accepted as altogether reliable.

Because of the paucity of data on the myxomycetes it is necessary to turn to studies of other types of organisms for light on the relations in two-membered cultures—the Acrasieae and the protozoa.

A thorough study of the relations between *Dictyostelium discoideum* Raper and the associated bacteria has been made by RAPER (**23, 24**). Although he found that *D. discoideum* is constantly associated with *Vibrio alkaligenes* Lehm. and Neum., it grew much better when certain other bacteria were used as associates. In two-membered cultures with bacteria the growth of the *Dictyostelium* was limited chiefly by the hydrogen-ion concentration of the substrate. *Dictyostelium* would not feed on those bacteria which raised the pH to a considerable degree; but if the increased alkalinity were prevented by buffering or using fermentable sugars, excellent development often occurred. The physical nature of the bacterial colony was also an important factor, for the *Dictyostelium* would not grow on bacteria which formed large amounts of slime, or which formed tough matted colonies (for example, *Bacillus mycoides*) difficult for the myxamebae to ingest. As with the myxomycetes, the feeding ability could not be correlated with morphological characters such as flagellation, spore formation, and Gram reaction.

The Gram reaction of the bacteria appears to be of little importance in determining the feeding behavior of the protozoa, although there is an impression that Gram-negative bacteria are in general more favorable as food than Gram-positive ones. This belief stems chiefly from the researches of OEHLER (**20**), who found that

of five amebae studied, one or two definitely preferred Gram-negative forms. In later papers (summarized in **21**) OEHLER found the ciliates, *Colpoda steinii* and *C. cucullus*, could be fed on a great variety of bacteria, refusing only the acid-fast ones. Furthermore, it was with the Gram-positive *Bacillus subtilis* that HARGITT and FRAY (**7**) obtained their best two-membered cultures of *Paramecium*, a finding supported by JOHNSON (**13**) and LESLIE (**17**). CLEVELAND (**3, 4**) also found, in general, better growth of the flagellate *Tritrichomonas fecalis* on Gram-positive bacilli than on Gram-negative bacteria; and as in OEHLER's work, only the acid-fast bacteria were refused (**4**). To the writer's knowledge, the acid-fast bacteria and the actinomycetes have never been successfully used in the cultivation of protists (**21, 4, 25**), although LESLIE (**17**) found a poor growth of *Paramecium* on mycobacteria.

Other factors seem to be more important than the Gram reaction. Slimy bacteria are not easily ingested either by myxomycetes, Acrasieae, or amebae (**5, 23, 6**). The metabolites of the bacteria are often important; thus highly putrefactive or fermentative bacteria are inimical (**7**), as well as those which produce certain nitrogenous substances, such as ammonia, urea, and trimethylamine. By using a medium in which these substances are not formed (**20**), however, it can be shown that the bacteria are not intrinsically unsuitable for food. KIDDER and STUART (**15**) claim that the pigments of certain chromogenic bacteria are toxic for the ciliate *Colpoda*, and HETHERINGTON (**9**) has shown that as marked differences can exist between the strains of a particular species as between genera.

It is clear that there are divergent results and opinions on the myxomycetes and other bacterial feeders in their relation to the bacteria. The present paper attempts to clarify these relations. Myxomycete plasmodia possess the great advantage of being macroscopic, and many of the changes taking place in two-membered cultures are therefore easily observed.

Material and methods

Two media were used mainly in these experiments, an oatmeal agar similar to the OA previously described (**5**) but prepared more exactly, and a yeast-extract-starch agar (YSA). The oatmeal agar was prepared by placing 0.5 gm. of oatmeal and 20 cc. of agar buffered at pH 6.0 (6.0 UA of **5**) in each 125 cc. Erlenmeyer flask, which was then plugged and autoclaved. The YSA was prepared by adding to the 6.0 UA 0.5 per cent Difco yeast extract and 2 per cent soluble starch. After the starch and agar had been dissolved by heating, 20 cc. of the medium was placed in each 125-cc. flask and the flasks plugged and autoclaved.

The plasmodia chiefly used were those of *Badhamia foliicola* List., strain 13, and *B. magna* Peck, strain 51. In addition, the following were used in several experiments: *Badhamia* sp., strain 11; *B. utricularis* Berk., strain 22; *Didymium*

squamulosum Fr., strain 14; *Physarum polycephalum* Schw., strain 24; and *Stemonitis axifera* MacBr., strain 21.

In most of the experiments it was desired to use comparatively large inocula of as uniform size as possible; and in view of previous observations of the physiological differences between plasmodia of the same species grown under different conditions, it was further desired that all inoculations of a single species be made from a single stock plasmodium. After some experimentation, it was found that large, pure-culture plasmodia for inoculation could be grown on autoclaved yeast (YA) in large Roux flasks (**5**). One hundred and fifty cc. of agar buffered to a pH of 6.0 (6.0 UA; **5**) was placed in each flask and the flasks plugged and autoclaved. After autoclaving, the flasks were laid horizontally until the agar had solidified. Excessive condensation of water was prevented by drying the flasks in an incubator for several days.

To each flask 1.5 cc. of YA was added under sterile precautions, the yeast suspension distributed over the agar, and each flask inoculated with as large a pure culture inoculum of the particular plasmodium as was feasible. The flasks were incubated horizontally at 25° C. until most of the yeast was consumed and then stored at 12° C. until needed. With experience, large plasmodial fans of about equal thickness throughout could be obtained; and by removing plasmodial fragments of equal area, approximately equal quantities of inoculum were obtained. For this purpose a punch-cutter was employed, made of a short length of nichrome tubing of 5 mm. internal diameter riveted to a brass handle. The cutting edge of the tube was slightly beveled on the exterior side for easier cutting. The punch was flamed, introduced into the flask and cooled, and then as many cuts made as necessary. The material was transferred to the culture vessels by means of a flattened nichrome needle.

After autoclaving to remove the agar, the dry weights of the inocula were obtained by placing the coagulated plasmodia on weighed cover slips and drying to constant weight. The weights thus obtained were between 0.1 and 0.2 mg. in almost all samples.

The bacteria[2] were suspended either in yeast-extract broth (YE, **5**) or in yeast-extract-dextrose (YED, **5**) and incubated at least 48 hours at 25° C. before use. They were then inoculated into the flasks of OA by means of Pasteur pipettes. Not more than two or three drops were employed, and the inoculum was dropped directly on the plasmodium and surrounded it. If the plasmodia were not well infected, they would move away from the point of inoculation, leaving the bacteria behind with consequent delay in feeding and growth.

[2] The bacteria were obtained from a number of sources and are now deposited with the Laboratory of Plant Physiology of Harvard University, under identifying strain numbers.

Results

Bacteria associated with plasmodia

In view of statements that several species of bacteria were found in constant association with different plasmodial species of myxomycetes and Acrasieae (**22, 23, 26, 28**), a qualitative analysis of the microbial flora of gross cultures of plasmodia was undertaken to determine the significance of their association.

TABLE 1

Name or isolation number of bacteria	Myxomycetes				
	Badhamia sp.	B. foliicola	B. utricularis	Physarum polycephalum	Stemonitis axifera
I*	+				
II	+				
III	+				
IV	+				
V		++	+++	+++	+++
Ps. schuylkilliensis		+++	+++	+++	+++
Ps. annulata		+			
VIII					+++
IX					+
X					+
Ps. ovalis			+		+
XII					+
XV			+		
XVI			+		
XVIII				++	
B. fulvum				+	
XXI				+	
XXII			+		
B. helvolum			+	+	
B. coli		+		+	
XXIX	+++	+++	+++	+++	

* Characters of unidentified forms.—Gram negative and cephalotrichous: I, II, III, IX, X, XV, XXI, XXII. Peritrichous: V. Gram positive and motility undetermined: IV (?), XVI. Liquefy gelatin: I, II, V, VIII, X, XII, XV, XVI, XXI, XXIII; others do not. Surface growth in yeast-extract agar stab: III, IV, VIII, IX, XXII; others deep growth as well (X, XII, XXIII show only slight deep growth).

The six plasmodia used (with the exception of *Didymium squamulosum*) had all been grown in gross culture on OA for some time. Gross culture sclerotia were placed on OA of pH 6.0 in large moist chambers and kept in diffuse light at 25° C. until most of the plasmodia had fruited, thus demonstrating favorable conditions for completion of the life cycle. Suspensions were prepared from these cultures, plated on the same medium, and incubated in the dark at 25° C. for 5 days. Twenty-five distinct colonies were obtained, which were examined for purity and subsequently cultured and stored on YE or on YED. Table 1 shows the characters of the bacteria and their relative frequency (as indicated by the number of + signs)

with each myxomycete. Duplicate isolates and bacteria of adventitious origin are omitted from the table. In addition to the bacteria listed, there are two species not isolated as just described. No. XXIII was isolated from a culture of *Didymium squamulosum* and *Saccharomyces ellipsoideus* on plain agar. It appeared to be the only bacterial species present. No. XXVIII, *Bacillus pabuli* Schiebl., a starch-hydrolyzing bacillus, was found as a contaminant in oatmeal cultures when the oatmeal was insufficiently autoclaved.

Table 1 indicates that these bacteria are almost all Gram negative, the majority liquefy gelatin, and most of them are motile. Their growth shows nothing very characteristic, and they are—superficially at least—referable to those two great heterogeneous groups of common soil and water bacteria, "*Achromobacter*" and "*Flavobacterium*." It should be noted further that, with the possible exception of the associate of *D. squamulosum*, no one of these bacteria was constantly and exclusively associated with a particular myxomycete; those which were strikingly abundant on one isolation plate were also abundant on several. *D. squamulosum* was not grown in the same manner as were the other organisms, and the conditions in a *S. ellipsoideus* enrichment plate (**5**) may well have allowed the development of a single bacterium to the exclusion of others.

Preliminary experiments showed that all but a very few of these bacteria formed good two-membered cultures with myxomycete plasmodia. In view of the fact that there were no constant associates, the attempt to study these associations was abandoned. Instead a study was made of the range of known bacterial forms on which the myxomycetes could feed.

Studies with known organisms

As pointed out in the literature review, most general rules determining the ability of protists to feed on bacteria according to "natural" groupings have sufficient exceptions to invalidate them or else insufficient data to support them. It was desirable, therefore, to determine whether any similar generalities could be made for the myxomycetes, and for this purpose a division of the bacteria into reasonably consistent natural groups was necessary. The manuals and systems of bacteriology extant are either out-of-date or else form groups which are hardly consistent (**1**). These classifications have been discussed by Kluyver and van Niel (**16**), who proposed an improved, simplified system. A number of known strains of bacteria were collected to represent as far as possible the tribes and genera defined by these writers. These bacteria and several of the determined species isolated from myxomycete gross cultures were used for further study.

The bacteria and the two myxomycete plasmodia, *Badhamia foliicola* strain 13 and *B. magna* strain 51, were inoculated, each in duplicate, into OA flasks in the

manner described under methods. The results of this experiment are summarized in table 2 for *B. magna*.

Because they grew little if at all on the ΘA, and furnished no nutrient for the plasmodia which likewise did not grow, the following bacteria used in these experiments are excluded from table 2: *Micrococcus* (*Staphylococcus*) *aureus* Migula, *M.* (*Staph.*) *citreus* Migula, *Sarcina lutea* Schroeter, *Sporosarcina* (*Sarcina*) *ureae* Beij., *Betacoccus arabinosaceus* Orla-Jensen, *Bacterium* (*Cellulamonas*) *liquata* McBeth and Scales, and *Zymobacillus macerans* Schardinger.

I. Natural groups of bacteria in relation to feeding.—Table 2 shows that there is no general correlation between the tribes and genera of these bacteria and the ability of the plasmodia to feed. There is but one group characteristic which limited the growth of the plasmodia, and that is the very limited growth on OA of several of the cocci: the Micrococcaceae (*M. citreus*, *M. aureus*), the Sporosarcineae (*S. ureae*), the Sarcineae (*S. lutea*), and the Streptococceae (*Betacoccus arabinosaceus*). *Micrococcus albus* and *Micrococcus* sp., which grew fairly well on this medium, also supported fair to good growth of the plasmodia.

The acid-fast bacteria, *Mycobacterium* spp., *Proactinomyces* sp., and *Actinomyces* sp., supported fair to excellent growth of the plasmodia, although these forms have been found unsuitable for the cultivation of other protists (**21, 25, 4**). The Gram-positive Bacilleae (fig. 1*A*), with the exception of *B. subtilis* and the Gram-positive *Kurthia zopfii*, supported as good growth as members of the Gram-negative genus *Bacterium*.

II. Physical nature of bacterial colony in relation to feeding.—The physical nature of the bacterial colony often determines the ability of the plasmodia to feed. Thus the slimy capsules formed by several bacteria markedly inhibit feeding (table 2). The young colonies of some bacteria are rather slimy or moist (for example, *Ps. annulata*) but later become more or less dry, with correspondingly increased ease of attack by the plasmodia. The feeding on dry compact colonies differs characteristically from that on moist slimy ones. The plasmodium attacks dry colonies by crawling over them as a fan, and the colony may often be seen through the transparent layer of myxomycete protoplasm. This the writer has called central feeding (table 2), since the plasmodium may cover the colony to the very center. On the other hand, in the peripheral feeding on slimy colonies, the plasmodium banks up against the edge of the colony without penetrating or covering it. The plasmodium is more or less immobilized, and growth is exhibited as a gradual extension of the protoplasm around the edge of the colony from the initial feeding point.

Growth is generally much slower on slimy colonies than on relatively dry ones; but if the plasmodium establishes itself, it may finally creep over and destroy the colony entirely. In such a case the fan is irregular, the strands of plasmodium be-

TABLE 2

BACTERIA (TRIBE, SPECIES, STRAIN NUMBER)	GROWTH OF PLASMODIA	TAXIS TOWARD COLONY	LENGTH OF LIFE (DAYS)	TYPE OF FEEDING	FINAL pH OF CULTURE	CONDITION OF PLASMODIA AT END OF CULTURE
Pseudomonadineae						
Pseudomonas annulata						
Ches. Ps2	+	+	6	p†	4.5	Dead
	+++	+	40	p	7.6	Dead
Ps. schuylkilliensis Ches.						
Ps3	+++	+	27	c‡	.....	Sclerotized and dead
	+++	+	27	c	8.0	Sclerotized and dead
Rhizobium trifolii Dang.						
Rh1 *	+	−	6	p	4.9	Dead
	+	+	6	p	5.2	Dead
Micrococcaceae						
Micrococcus albus Buch.						
Mcc3	++	o+	54	c	7.6	Dead, dissociated
	++	+	40	c	7.1	Dead
Micrococcus sp. Mcc1	+++	+−	40	c	6.1	Dead
	+++	+−	40	c	7.6	Dead, dissociated
Mycobacterieae						
Mycobacterium sp. Mc1	+	−o	15	c	5.3	Dead
	+++	−o+	40	c	5.6	Sclerotized, dissociated
Mycobacterium sp. Mc2	+++	o+	54	c	6.5	Living
	+++	+	54	c	6.5	Living
Bacterieae						
Kurthia zopfius Trev.						
K1	+++	+−+	40	c	3.8	Dead
	+++	+	54	c	5.9	Living
Bacterium acidilactici						
Mg. Bt6	+++	+	54	c	4.2	Dead
	+++	+	40	c	7.4	Dead
B. ceramicola Lundestad						
Bt3	++	+	40	c	6.0	Dead, dissociated
	++	+	40	c	3.9	Dead, dissociated
B. coli (form) Esch. Bt2	+++	o+	40	c	8.3	Dead, dissociated
	+++	+	40	c	8.3	Dead, dissociated
B. prodigiosum DeT Trev. Bt4	++	o+	40	p	6.3	Sclerotized
	.....	.....	6	.....	7.4	Dead
Bacilleae						
Bacillus cereus Frank.						
Frank. Bc3	++	+	9	c	4.8	Dead
	+	+	54	c	3.9	Living, dissociated
Bac. danicus Löhn. West.						
Bc1	+	+	54	c	4.0	Living, dissociated
	+++	+	27	c	5.2	Living
Bac. megatherium De Bary Bc5	+++	+	40	.....	6.1	Living
	++	+	54	.....	3.9	Living, dissociated
Bac. mycoides Flügge						
Bc4	++	+	40	c	3.9	Living, dissociated
	++	+	40	c	3.9	Living, dissociated

* Slimy colony. † Peripheral feeding. ‡ Central feeding.

TABLE 2—*Continued*

Bacteria (tribe, species, strain number)	Growth of plasmodia	Taxis toward colony	Length of life (days)	Type of feeding	Final pH of culture	Condition of plasmodia at end of culture
Bacilleae—*Continued*						
Bac. pabuli Schiebl. Bc2	+	−	15	.....	6.7	Dead
	++	o−	27	.....	7.4	Dead
Bac. subtilis Praz. Bc6..	.....	+−	15	o	.....	Dead
	.....	+−	15	o	.....	Dead
Aerobac. polymyxa Praz. Abc1*.....	++	+	54	p	.....	Dead
	+++	+	40	p	6.8	Dead
Actinomycetales						
Proactinomyces sp. PrAc1.....	.....	−	6	.....	5.0	Dead
	++	+	6	c	4.6	Dead
Actinomyces sp. Ac1...	+++	o+	27	c	7.5	Dead
	+	+	13	c	7.9	Dead
Undetermined						
Capsulated coccus X2*.	+++	o+	40	p	7.9	Dead
	+++	o	40	c	6.8	Dead
Capsulated bacterium Bbt1*.....	+	+	40	p	7.0	Dead
	+	+	40	p	6.9	Dead

ing twisted and lax (fig. 1*B*), having much the same appearance as a plasmodium on too moist a substrate. Contrasted with this is the "normal" appearance of a plasmodium (the fan flat), smoothly rounded at the front edge, and gradually breaking up into long, regular main strands converging at the rear.

It was thought at first that the dry, tough, nonwetting colonies of the actinomycetes and the dry, tough, adherent colonies of *Bacillus mycoides* would not be attacked, or at least only with difficulty. On the contrary, *B. mycoides* in association with the plasmodia furnished an excellent example of central feeding, and the actinomycete colonies were also completely eaten (fig. 1*D* II).

III. Tactic behavior of plasmodia and feeding.—Tactic behavior toward bacterial streaks was used by Watanabe (**29**) as a criterion of the ability of plasmodia to feed on bacteria. The plasmodia, placed on nonnutrient agar, were encircled with a streak of the bacterium to be tested. The suitability of the bacterial food was determined by noting whether or not the plasmodium followed the streak and fed or crossed it without feeding. In this connection, note the tactic behavior of the plasmodia toward the bacteria listed in table 2. Here a positive chemotaxis is indicated by +, a negative taxis by −, and no definite taxis by o.

A plasmodium often moved away from the point of inoculation, then by the third day returned to it (after the bacteria had begun to grow), left again, and again returned. Often there seemed to be no very definite taxis, but the plasmodi-

um wandered around until it came in contact with the colony and began feeding. Sometimes a plasmodium temporarily ceased feeding. In many instances feeding began only because the colony had spread to such an extent that the plasmodium could not very well move about without coming in contact with it.

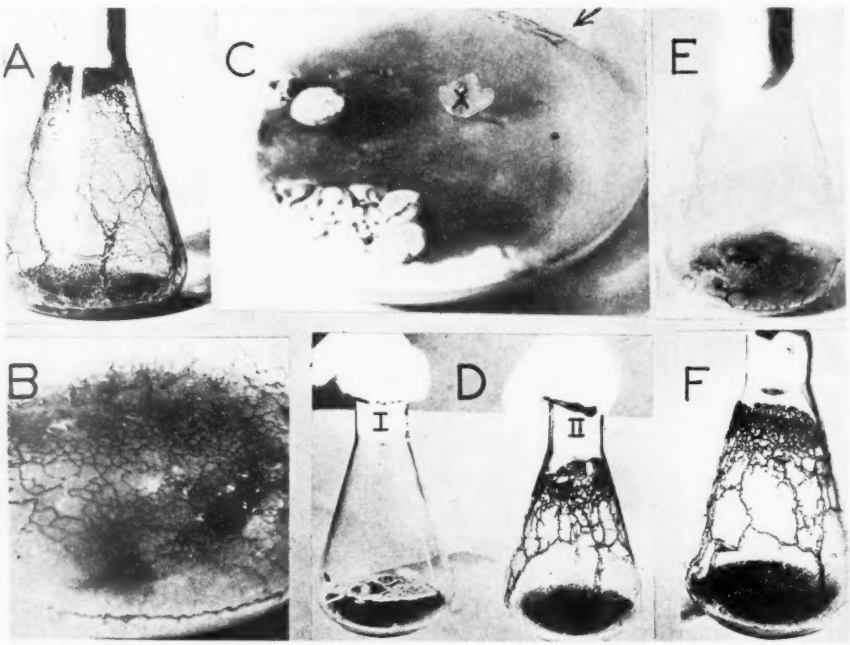

FIG. 1.—*A*, *Badhamia foliicola* on Gram-positive *Bacillus danicus*, oatmeal agar, 27 days' growth; plasmodium sclerotizing at top of flask. *B*, same on unidentified slime-forming bacterium (Bbt1), oatmeal agar, 20 days' growth. *C*, *Badhamia magna* and *Actinomyces* sp. on yeast-extract-starch agar: *Actinomyces* colonies in foreground; plasmodium inoculated at *X;* arrow points to starving plasmodium; 4 weeks after inoculation. *D*, *B. foliicola* and *Actinomyces* sp., oatmeal agar, 46 days after inoculation: I, plasmodium not in contact with colonies, starved; II, plasmodium cleared *Actinomyces* from agar, now sclerotizing. *E*, dissociation of *B. foliicola* in two-membered culture with *Mycobacterium* sp. (Mc1); 27 days' culture, dissociant beginning growth. *F*, same as *E;* 41 days' culture.

Although WATANABE (**29**) found that not one of seventeen different myxomycetes would ingest *Bacillus megatherium* on plain agar, the writer has shown that when they are forced to live in unavoidable contact with it, *Badhamia foliicola* and *B. magna* will feed on this bacterium.

A similar and even more striking example of the independence of taxis and feeding is found in the association of *Physarum polycephalum* with two species of yeast, *Saccharomyces ellipsoideus* Hansen and *Torula aclotiana* Kufferath. Although the

plasmodium was successfully established in two-membered culture with these yeasts, it would still feed on neither when streaked on plain agar plates. There are, of course, exceptions to the independence of chemotaxis and feeding. Most of the plasmodia in culture show pronounced positive chemotaxis to *S. ellipsoideus* streaked on plain agar; this fact was used to advantage in the enrichment technique (**5**).

When a plasmodium was transferred to a fresh substrate, it almost invariably and immediately left the old portion transferred with it for the new. In two-membered cultures on OA the plasmodium generally stopped crawling to erode the first grains with which it came in contact. This movement was slowed down so much that the bacteria with which the plasmodium had been inoculated grew faster than the plasmodium could rid itself of them by migration.

The first cultures were made on YSA because of its advantages over OA. Being nonparticulate, the YSA could be rendered wholly liquid by autoclaving, the coagulated plasmodium removed, and its dry weight obtained as a quantitative expression of growth. Furthermore, the plasmodium hydrolyzed the starch of this medium to simple sugars utilizable by the bacteria. Since the degree of hydrolysis was dependent on the quantity of plasmodium, the richness of the medium was controlled to some extent by plasmodial growth, and the danger of the plasmodium being swamped by heavy growth of the bacteria was lessened. But the several advantages of YSA were outweighed by one very serious disadvantage: the plasmodium crawled away from the inoculum so speedily that it freed itself of the bacteria in the process. In the absence of a well-defined positive taxis toward the colony growing at the site of inoculation, the plasmodium starved with only a few centimeters separating it from its bacterial food (fig. 1*C*). These cultures on YSA were made with *Badhamia magna* and all the bacteria of table 2.

Eleven of these cultures were selected at random and the myxomycete plasmodia from them inoculated into YED broth tubes. All except one remained sterile, confirming the statement that the myxomycetes had freed themselves of the bacteria by crawling away from the point of inoculation. The possibility remained that the immediate growth of many of the bacteria had produced toxic substances in sufficiently large concentration to inhibit feeding. Another group of twenty-five cultures was selected which included most of the bacteria of table 2, and the myxomycete reinoculated adjacent to each colony. The colony at the same time was spread around the myxomycete so that there would be no chance of the plasmodium avoiding constant contact with the bacteria. The myxomycete grew in eighteen of these twenty-five flasks, and on the same bacteria as in the OA flasks (fig. 1*D*); therefore toxicity of the bacteria could not have been a factor.

The factors influencing growth in two-membered cultures were sufficiently numerous as soon as a plasmodium, a substrate, and a bacterium were brought to-

gether, without adding the complication of the myxomycete freeing itself of its associate, as in most of the cultures on YSA. For this reason OA was adopted as a substrate, as on it the plasmodia, on leaving the site of inoculation, generally stopped creeping and started to erode those oatmeal grains with which they first came into contact.

IV. CHEMICAL SUBSTANCES ELABORATED BY BACTERIA.—Evidence was obtained that metabolites produced by the bacteria may be inhibitory for growth. *Bacterium acidi-lactici* and *Badhamia foliicola* were inoculated into flasks containing media of the following compositions:

A.	yeast extract	0.25%
	dextrose	2.0%; original pH 5.4
	agar	2.0%; final pH 4.1
B.	yeast extract	1.0%
	dextrose	2.0%; original pH 5.7
	agar	2.0%; final pH 5.0
C.	yeast extract	1.0%
	dextrose	0.5%; original pH 5.8
	agar	2.0%; final pH 7.9

At the end of 3 weeks there was excellent growth in the flasks containing medium A, but none in the flasks of media B and C. Because of its high final pH, no just comparison may be made between medium C and the other two. However, the final pH of medium A or B is within the range in which *Badhamia magna* grows well, and growth was inhibited solely by quadrupling the concentration of yeast extract. This concentration is not itself toxic to *B. foliicola*, and therefore lack of growth and death must have been due to metabolites elaborated by *Bacterium acidi-lactici* from the nitrogenous yeast extract.

Bacterium prodigiosum allowed a fair growth of both *Badhamia foliicola* and *B. magna*, although it is the same strain that KIDDER and STUART found extremely toxic for *Colpoda* and *Daphnia* (**15**). Nor did the plasmodia take up the colored granules sufficiently to change their natural color, in contrast to the observations of KAMBLY (**14**) on the plasmodia of several myxomycetes, and of RAPER (**23**) on the myxamebae of *Dictyostelium discoideum*.

V. DISSOCIATION OF PLASMODIA.—Another phenomenon in culture somewhat parallels the adaptations mentioned elsewhere (**5**). Because the phenomenon resembles to a great degree the dissociation of bacterial species in which a colony of bacteria gives rise to a variant colony differing in certain respects from the parent, the same name is applied.

In several of the cultures the plasmodia started growth and then fragmented into several smaller plasmodia, one or more of which grew abundantly, while the others did not feed and finally died or were lost in the greatly increased mass of the

"dissociants." Plasmodia have also been observed to grow fairly well in two-membered cultures and then apparently to die, while much of the bacterial mass remained unconsumed. Close inspection revealed that there were minute living plasmodial fragments left, and these dissociants began growth anew in the same culture and on the same bacterial mass (fig. 1*E*). The final growth sometimes surpassed the original, the entire mass being consumed (fig. 1*F*) and the oatmeal grains completely hollowed. The vegetative state ended in most cases by the plasmodium sclerotizing on the walls of the flask. These dissociations have been noted in *Badhamia foliicola* feeding on *Micrococcus albus*, *Bacillus megatherium*, and *Mycobacterium* sp. (Mc1); and in *B. magna* on *Micrococcus albus*, *Micrococcus* sp., *Mycobacterium* sp. (Mc1), *Bacterium ceramicola*, *B. coli*, *Bacillus cereus*, *Bac. danicus*, *Bac. megatherium*, and *Bac. mycoides*.

Interrelations between bacteria and plasmodia

Under this heading are placed those phenomena in culture not readily assignable to the bacteria or to the plasmodia but to an interaction between both.

I. Hydrogen-ion concentration of medium.—It was stated earlier (5) that the myxomycete plasmodia did not grow on those bacteria which raised the pH to an unfavorable degree (above 7.4). Yet in these later studies the final pH of two-membered cultures in which there was excellent development of the plasmodium was often extremely high (8.3 for *Badhamia magna* on *B. coli*). Furthermore, duplicate cultures may in certain cases have widely different final hydrogen-ion concentrations (table 2). Often within the same culture the pH varied from place to place, being generally comparatively high near the bacterial colony, low near a portion of a living plasmodium not in contact with the colony, and high near any mass of dead plasmodium. The single values given in table 2 were all taken near the plasmodia, preferably living ones if they were alive when the cultures were discontinued.

Since the quinhydrone electrode was used for these determinations, it is possible that the potentials were the combined expressions of hydrogen-ion activity and oxidation-reduction activity, were it not that determinations made with a glass electrode and with acid-base indicators were in thorough agreement with the quinhydrone determinations.

An analysis of the changes of pH produced in the substrate by the plasmodium alone was necessary, and examples are given in table 3. These support the conclusion that in presence of carbohydrates, the living, active, plasmodium lowers the pH. After death, presumably through the autolysis of its nitrogenous components, it raises the pH.

Thus, it might be expected that since both the plasmodium and the bacterium affect the pH of the medium, the relative sizes of the two inoculations would in-

fluence the final pH of a culture. Experiments with *Badhamia foliicola* and *Pseudomonas schuylkilliensis* confirm this supposition. *Ps. schuylkilliensis* inoculated into flasks of OA raised the pH of the medium to 7.4 in about 3 days. Small (0.5×1 mm.) inoculations of *B. foliicola* into these cultures died immediately. In the next experiment, larger inoculations of the *Badhamia* were made into the flasks and the quantity of added *Pseudomonas* suspension reduced. The plasmodia began growth, but were soon outstripped by the *Pseudomonas* and died in a week. In the final experiment, given in table 2, the plasmodia were inoculated in relatively large quantity compared with the *Pseudomonas*, and their feeding limited the accumula-

TABLE 3

CHANGE IN PH OF OATMEAL AGAR AFTER GROWTH OF PLASMODIA; ORIGINAL PH 5.9–6.1

CULTURE	FINAL PH	REMARKS
Badhamia foliicola (pure) on oatmeal agar...	5.4	Small (starving) plasmodium
B. foliicola on oatmeal agar+autoclaved yeast	5.8	Plasmodium recently dead; pH taken adjacent plasmodium
Same culture	5.5	pH taken at distance from plasmodium
B. foliicola on oatmeal agar+autoclaved yeast	4.8	Plasmodium alive; pH taken adjacent plasmodium
Same culture	5.6	pH taken at distance from plasmodium
B. magna on oatmeal agar, two-membered culture with Sacch. ellipsoideus	6.9	Good growth; plasmodium dead, disintegrated
S. ellipsoideus alone on oatmeal agar	4.8	Thirty days' growth

tion of two great numbers of bacteria, with the result that the plasmodia grew until the nutrients of the culture were exhausted.

As in previous experiments (**5**), it was found that plasmodia inoculated immediately from a medium of pH 6.0 into media of pH 7.4 or higher promptly died. If the plasmodia were transferred successively through media, each of a slightly more alkaline reaction than the preceding, they could live and grow at a final pH of 7.4. The same adaptation probably operates in two-membered cultures in which plasmodia have grown with alkali-forming organisms. The cultures listed in table 2 were kept until the plasmodia had died, sclerotized, or fruited in most of them (some as long as 2 months). During this time, in which a balance existed between the myxomycetes and the bacteria, adaptations to the slowly changing conditions could easily have occurred.

II. CARBOHYDRATE CHANGES IN MEDIUM.—It was found previously (**5**) that myxomycete plasmodia in a pure state on OA could erode the grains, but without growing appreciably, and it was concluded that bacteria or other microörganisms

were necessary for complete utilization of the medium. The more extensive erosion occurring in two-membered cultures offered two possibilities: (*a*) the myxomycete could utilize the oatmeal as an accessory nutrient provided bacteria or yeasts were present; or (*b*) the microörganisms themselves could act on the medium in such a manner that it could then be utilized by the myxomycete.

It was found that the yeasts and the majority of the bacteria cultivated on OA could not erode the grains exposed at the surface. An exception was the bacterium isolated from oatmeal and identified as *Bacillus pabuli*. Yet the plasmodia grew equally well, and in many cases better, in two-membered culture with microörganisms which alone had no effect on the grains. That the bacteria did not contribute something to the medium which would allow growth in pure culture, and that they did not prepare the starchy grains so as to make them more easily assimilated, was demonstrated by the following experiments.

Bacterium coli was grown in pure culture on OA and the cultures divided into three lots: one lot was autoclaved and shaken to distribute the bacteria throughout the medium; another lot was heated to 60° C. to kill the bacteria; a third lot was heated to 60° C. and the bacteria carefully washed off the surface with sterile water. *Badhamia foliicola* was inoculated into all three series of flasks. In those lots in which the bacteria were autoclaved and suspended throughout the medium, and in those where they had been killed by heating to 60° and washed off, there was no growth and little erosion of the starchy contents of the grains. In the third lot, in which the bacteria remained on the surface and were easily accessible, the plasmodia grew, devoured the heat-killed bacteria, and eroded the surface grains extensively.

An experiment with autoclaved yeast confirmed this result. The yeast was added to flasks of OA, 0.5 cc. per flask, and the flasks agitated to distribute the suspension over the surface. Equal-sized inocula of *Badhamia foliicola* were placed in each flask and in control flasks of OA alone. The flasks were incubated until the yeast had been completely consumed. In the control flasks, in which the plasmodia were minute and starving, the average number of grains eroded was seven; in the flasks containing YA, in which there was a fair growth of the plasmodia, the average number eroded was twenty.

It seemed that the disappearance of the starch as indicated by erosion of the grains was proportional to the size of the plasmodium, and this was confirmed by inoculating pure culture plasmodia ranging in size from minute inocula to those covering an area of several square centimeters into OA flasks. The number of eroded and hollowed grains varied roughly with the size of the plasmodia.

The digestion of cooked starch by plasmodia had been observed by LISTER (18) and was confirmed by the experiments just described. But in addition, the writer noticed that some microörganisms, particularly yeasts, when grown on a starchy

medium such as oatmeal or potato agar were benefited by the presence of a plasmodium.

It was evident that not only did the yeast support growth of the plasmodium, but the plasmodium also supported growth of the yeast. Neither plasmodia killed by heating and placed in flasks of OA, nor extracts of plasmodia, produced better growth of the yeast than did the OA alone. Therefore the myxomycetes were changing the medium to a more nutritious one for the yeast. These observations were confirmed by pouring suspensions of living yeast cells (*S. ellipsoideus*) in properly buffered YSA into petri dishes. No growth of the yeast occurred, for the essential sugar was absent. On introduction of any one of the plasmodia obtained in pure culture (**5**, table 1) there was growth of yeast in the track of the plasmodium and concomitant disappearance of the starch, as shown by the iodine test.[3]

Within a two-membered culture an ecological balance is maintained between both members, provided care is taken that neither member is introduced in so large quantity that it overwhelms the other. This balance is shown by an opposing effect of the plasmodium on the raising of the pH by bacteria, and in a cycle wherein the plasmodium begins to feed on the microörganism, at the same time breaking down starch into simple sugars, which promote growth of the microörganism so that it is further used by the plasmodium, the cycle continuing until the substrate is staled or exhausted.

Discussion

It has been shown that in undisturbed gross cultures of myxomycete plasmodia there are several common bacteria which dominate the flora. However, in contrast to the statement of SKUPIENSKI (**26**) that each plasmodium has its characteristic bacterium, a bacterium common in one culture is also common in several. A seeming exception—the association of bacterium XXIII with *Didymium squamulosum*—has been discussed earlier.

The gross cultures of plasmodia other than *D. squamulosum* were grown on OA, which may be considered also as a specialized medium because of its high carbohydrate and low protein content. The medium was buffered at pH 6.0; the temperature was approximately 25° C. Thus those bacteria which require rather restricted conditions for growth (that is, higher temperature), a medium rich in proteins, and greater alkalinity, were excluded in favor of the organisms which could grow better under these conditions. These bacteria, as might be expected, were the common Gram-negative pseudomonads and non-spore-forming peritrichs typical of water and moist plant remains. It seems apparent, therefore, that the cultures were enrichment cultures for these bacteria as well as for the plasmodia, and that the single criterion of a more or less constant association is not sufficient to

[3] Data confirming the elaboration of a diastase by plasmodia will be given elsewhere.

postulate a symbiosis. If there were any closer relationship than this, it would be expected that the plasmodia could not grow at all well with organisms other than their natural associates. Indeed the opposite is true, for two-membered cultures could be made with astonishingly diverse microörganisms, including the yeasts, acid-fast bacteria, and actinomycetes. These facts are supported by the demonstration of HOWARD and CURRIE (**11, 12**), who showed that *Physarum polycephalum* can feed on a number of fungi other than those with which it is naturally found.

A much better case could be made for symbiosis in the two-membered cultures. First, it has been shown that under certain conditions the shift of the hydrogen-ion activity is kept from going too fast in one direction or the other; and second, the extracellular digestion of the starch by the myxomycete results in the enrichment of the medium for those second members which can utilize sugar, the increased growth of the second member being then reflected in an increased growth of the myxomycete.

Nevertheless the writer does not consider a two-membered culture to be a symbiosis. It seldom happens that the plasmodium sclerotizes or fruits with much of the bacterial mass uneaten. If the bacteria are not cleared, the plasmodium generally dies. But if the plasmodium does grow abundantly, it is at the final expense of the bacterium whose colonies are completely cleared from the medium. Whether or not the myxomycete enriches the medium for the bacterium depends on the bacterium and the medium. If sugar is supplied for an organism that can utilize sugar, there is no need for the starch-destroying power of the myxomycete. If the microbe can easily dispense with sugar, as is the case with many pseudomonads, the starch hydrolysis is of no avail. Furthermore, the association is neither close nor constant and may be made with a plasmodium and any one of a number of very different organisms.

The complexity of the food relations and the difficulty of study are further enhanced by the adaptations mentioned previously (**5**) and by the dissociations found here.

The writer is not the first to report the breaking up of a plasmodium, for LISTER (**18**) recorded similar behavior in the case of *Badhamia utricularis* fed on the unfavorable *Agaricus rubescens* and *A. fascicularis*. The plasmodium in both cases fed to some extent, then broke up into portions, some of which died and others began feeding again.

HOWARD (**10**) also has noted *Physarum polycephalum* plasmodium breaking up into portions, some of which continued to feed and others to sclerotize or fruit. The writer has observed the same behavior with this and other plasmodia, and in addition has seen *P. polycephalum* in two-membered culture break up into two portions, the strands of which were interwoven without fusing. One portion later

sclerotized while the other continued to feed. SMART (**27**) has observed a closely related phenomenon in the case of *Fuligo septica*. The plasmodium of this species under certain conditions goes through several color changes, ranging from white to rusty yellow. White plasmodia will fuse with other white plasmodia and yellow ones will fuse with yellow, but cross fusions do not take place, although the plasmodia may be of the same clone.

Summary

1. In a study of myxomycete gross cultures, twenty-five different bacteria have been isolated, nearly all Gram negative, and most of them motile and possessing the property of liquefying gelatin. In the gross cultures on oatmeal agar there was found to be no specific association of a particular microörganism with a particular plasmodium, and those bacteria common in association with one plasmodium were common in association with several. These bacteria possessed the characters of the common soil and water bacteria, and it is concluded that there was no symbiosis between a bacterium and a plasmodium, but that the medium used was favorable to both.

2. A further study made with thirty-one different bacteria belonging to diverse groups showed no feeding preference of the plasmodia on the bases of morphological and natural groups. Unlike other known bacterial feeders, the plasmodia were found to grow on the mycobacteria and actinomycetes. The positive or negative chemotaxis toward the colony provided no indication as to whether the myxomycete can feed on the particular bacterium.

3. The final pH of the medium was controlled by both the plasmodium and the bacterium. If the pH of the medium were changed too speedily, the myxomycete could not grow. But by adjusting the medium and the relative sizes of the plasmodial and bacterial inoculations, the pH changed rather slowly and a balance could be obtained in which the plasmodium broke down the starches of the medium into sugars utilizable by the associated microörganisms, which grew more abundantly and in turn furnished more food for the plasmodium.

4. Only two factors were found which consistently limited growth of the plasmodium. The myxomycetes grew poorly on bacteria which formed slimy colonies and on bacteria which grew poorly on the media employed.

5. A phenomenon comparable with bacterial dissociations was found in some cultures, namely, the plasmodium grew to some extent, most of it then died, but small living fragments began growth anew.

The writer acknowledges his gratitude to Dr. WILLIAM H. WESTON, Jr., and Dr. KENNETH V. THIMANN of Harvard University for their stimulating and critical suggestions during the course of this work, and to Dr. C. B. VAN NIEL of the Hop-

kins Marine Station for his careful checking of the manuscript. To Dr. NATHAN R. SMITH of the U.S. Department of Agriculture for identifying many of the bacteria, the writer expresses his appreciation.

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DISTRIBUTION OF CALCIUM OXALATE CRYSTALS IN RICINUS COMMUNIS IN RELATION TO TISSUE DIFFERENTIATION AND PRESENCE OF OTHER ERGASTIC SUBSTANCES

FLORA MURRAY SCOTT

(WITH FORTY-TWO FIGURES)

Introduction

Ensheathed crystals of calcium oxalate in *Ricinus* were first described by ROSANOFF (**17**) in 1865. In the original figures the druses appear inclosed within distinct envelopes, supported approximately in the center of the cell by short stalks attached to the wall. In 1888, WAKKER (**23**) adopted the tonoplast method of DE VRIES and laboriously studied cells plasmolyzed to the point of protoplasmic rigidity by prolonged treatment with KNO_3 and eosin. He states that crystals always arise in vacuoles, and that stalks and sheaths are present only in cells devoid of living protoplasm and are therefore dead cell artifacts. WAKKER concludes: "Es wäre immerhin besser diese Balken als Rosanoff'sche Balken zu unterscheiden, und so werden wir die denn weiter auch nennen."

During the next 10 years discussion centered around the origin of crystals, whether in the cytoplasm or in vacuoles, and the nature of the crystal sheath, whether a dead cell artifact or a normal growth. WITTLIN (**24**) reviews this literature briefly and, in a survey of crystal formation in medicinal plants, outlines the development of crystals in *Ricinus*. The extensive literature of more recent years has been surveyed by MEYER (**8**) and NETOLITSKY (**10**), and the crystallography of calcium oxalate has been discussed in detail by FREY (**5**).

The mechanism of the origin of oxalic acid and calcium oxalate remains a problem for the phytochemist and the physiologist rather than for the anatomist. Plant anatomy, however, is concerned with the distribution of calcium oxalate throughout the living plant, since a study of distribution in space and time serves to throw light on the relation of crystal deposition to other dominant phases of tissue metabolism (**3**). As NETOLITSKY (**10**) points out, developmental studies in individual plants are curiously lacking.

The present paper is concerned with the development of calcium oxalate crystals and their distribution in the seedling and mature plant of *Ricinus* in relation to tissue differentiation and the presence of other dominant ergastic substances. Etiolated shoots and seedlings are also examined.

Material and methods

Ricinus grows freely throughout the entire year in southern California, and seedlings and mature plants are available at all seasons. Sections of fresh material from different regions—stem, root, leaves, inflorescence and fruiting axis, fruit and seed, seedling axis, and cotyledons—were examined under ordinary light, polarized light, and with dark-field illumination. Etiolated shoots and seedlings were also examined. Fresh material treated with 1 per cent osmic acid may be mounted in Berlese medium.[1] Fatty substances appear intensely blackened, calcium oxalate crystals stand out clearly, and the wall contraction inevitable in xylol-Canada-balsam preparations is avoided. In the detailed examination of the younger tissues, imbedded material is necessary, of course. Navashin's solution or osmic acetic solution[2] proved satisfactory.

Sections of varying thickness were examined unstained or after treatment with haematoxylin or with safranin and fast green. Ergastic substances were identified microscopically and by microchemical tests. They include (in addition to calcium oxalate) fatty substances, starch, tannin, sugar, the pigments chlorophyll and anthocyanin, and the wall materials cellulose, lignin, suberin, and cutin.

Observations

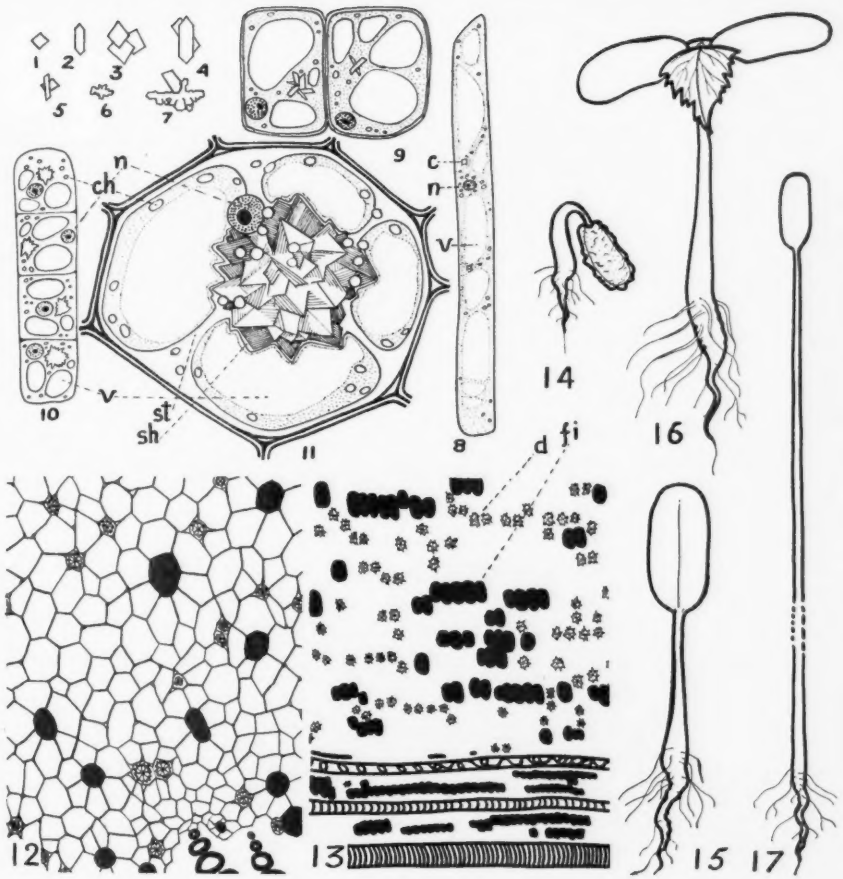
Types of crystals and their development

Two types of crystals are present in *Ricinus*, druses and solitary crystals. These conform to the crystallographic types described by Frey (5). The former are distributed throughout parenchyma tissues in general but are particularly conspicuous at the nodes, while the latter are practically restricted to the cells adjacent to the fibers of the secondary phloem.

Druses (figs. 1–11).—In the apical meristem of the shoot the first traces of crystal formation appear as minute points of light, visible only under the polarizing microscope. The earliest identifiable druses occur in the vacuolating cells of the third internode and in the leaf and bud sheath primordia of the apical region. Druse development is most readily observed in the leaf; in tangential sections of the projecting leaf veins, all stages of development may be found in one or a few sections. The druse originates as a single crystal, which may be cubical, bipyramidal, rodlike, or needle-like in form, and 2–3 μ in greatest diameter. Whatever the outline, the crystals in undamaged cells are suspended in protoplasmic strands in vacuolating cells. In this connection the penetration of neutral red is useful in defining the cell sap and vacuoles, and thus delimiting the protoplasmic strands.

[1] H_2O (distilled), 100 cc.; gum arabic, 60 gm.; chloral hydrate (saturated solution), 100 cc.; glycerin, 40 cc.

[2] Osmic acid (1%), 1 part; acetic acid (2%), 1 part.



FIGS. 1–17*.—Figs. 1–11, stages in druse development: figs. 1, 2, individual crystals; figs. 3–5, crystal aggregates; figs. 6, 7, dendritic growth; figs. 8–10, developing crystals within cell (8, two single crystals in protoplasmic strands; 9, crystal aggregates; 10, miniature druses from longisections of leaf veins); fig. 11, druse in mature parenchyma cell at petiole base. Figs. 12, 13, distribution of fat and calcium oxalate in pith of primary tissues, eleventh and twelfth internodes: fig. 12 (transection) and fig. 13 (longisection) show alternation in spaces of druses (stars) and fat idioblasts (solid black), circles, and ellipses. Figs. 14–16, stages in development of green seedling: fig. 14, hypocotylary arch stage, cotyledons inclosed in endosperm; fig. 15, straight hypocotyl, cotyledons green; fig. 16, plumule developing. Fig. 17, etiolated seedling; actual length 32 cm.

* Abbreviations: *c*, cambrium; *ch*, chloroplast; *cr*, crystal; *d*, druse; *f*, fiber; *fi*, fat idioblast; *n*, nucleus; *s*, starch; *sh*, sheath; *sp*, sieve plate; *st*, stalk; *v*, vacuole. All drawings with camera lucida.

If the protoplasmic strand is broken, the crystal may be released into the vacuole and Brownian movement is observed. The number of crystals in a cell is variable. As a general rule, in isodiametric cells one crystal only is present, somewhere near the center of the cell. In elongated cells two to four crystals may occur, distributed at approximately equal intervals throughout the protoplast.

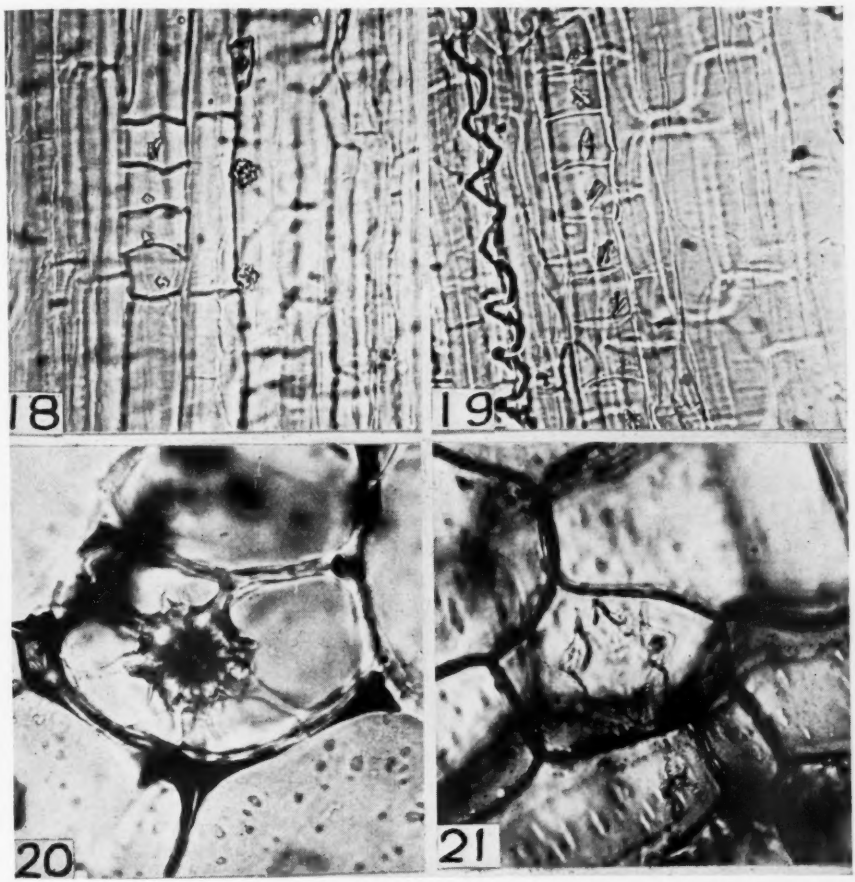
The further development of the druse is variable. An aggregate may appear, consisting of two or more similar or dissimilar individual crystals. On the other hand, dendritic growth appears to be equally common, and increase in volume takes place by haphazard accretions. This dendritic phase is soon masked by more regular crystal growth, and the miniature druses—a few microns in diameter—are exact replicas of the fully developed rosette crystal aggregates (figs. 18, 19).

The larger druses are inclosed within a cellulose sheath and anchored to the cell wall by one or more hollow cellulose stalks. It is impossible by current microchemical and microscopic methods to determine exactly at what stage cellulose deposition first begins. Small crystals about $3\,\mu$ in diameter already possess a tenuous sheath, demonstrable after careful solution of the crystal but not identifiable microchemically. Since crystal idioblasts and the inclosed druses increase in volume during tissue differentiation, the crystal sheaths and the stalks must be plastic in nature. Pectic substances and possibly some protein are therefore probably present in the original sheath and are masked by the later development of cellulose. The hollow stalk is presumably laid down around a protoplasmic strand. The strand itself is either retracted from the mature stalk or becomes indurated and persistent within it (figs. 20, 21). That cessation and resumption of crystal growth may take place is indicated by the occasional appearance of pitted cellulose sheaths. Crystal points may project beyond a heavy meshlike sheath, and suggest that localized breakdown of the original envelope has occurred, followed by renewed crystal growth.

When some of the larger druses are treated with IKI and H_2SO_4, followed by H_2SO_4CP, dissolution of the sheath is not complete. A brownish envelope remains which stains slightly with Sudan III and may therefore indicate sheath suberization. This suberization is erratic in occurrence and is not limited to certain regions nor to certain sizes of crystals. The wall of the idioblast itself remains unchanged, and shows—so far as observed—no trace of suberin or of lignin, as noted by WITTLIN (**24**) and others.

Young crystal idioblasts differ microscopically from ordinary parenchyma cells only in size, as will appear later, and in their crystal content. Nucleus, chloroplasts, mitochondria, and vacuoles are normal in number, appearance, and distribution in the protoplasts. As the crystal develops the nucleus may remain central in position, adjacent to the druse, or may retreat to the wall, generally adjacent to a stalk base.

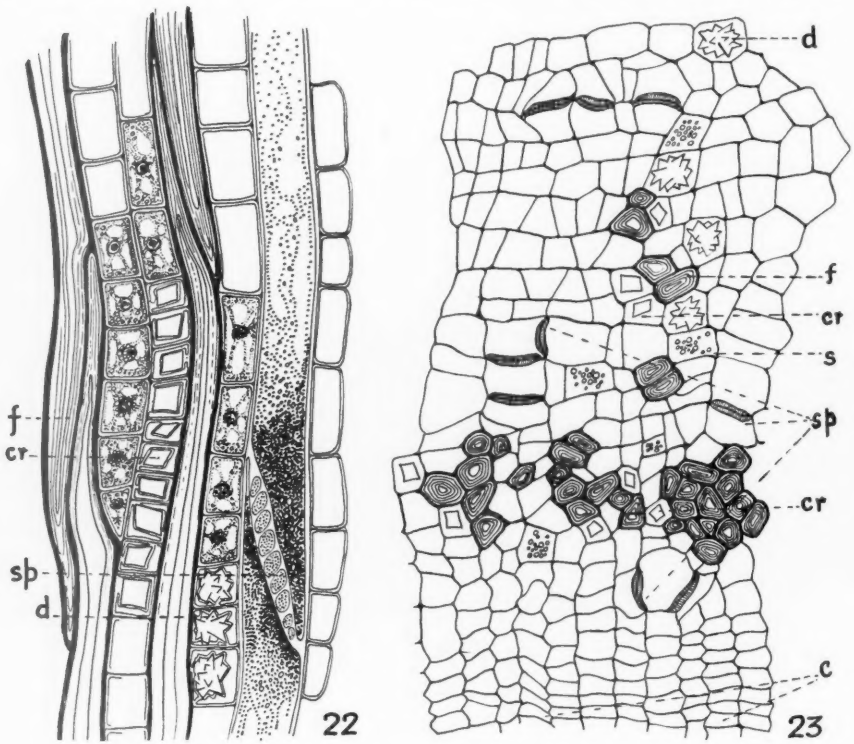
SOLITARY CRYSTALS (figs. 22, 23).—Solitary crystals are abundant only in the secondary phloem adjacent to the phloem fibers. They appear in vertical files of isodiametric parenchyma cells, which differentiate from cambial fusiform initials.



FIGS. 18–21.—Developing and mature crystals: figs. 18, 19, druse development, individual crystals, crystal aggregates, dendritic growth, and miniature rosettes from longisection of leaf vein; fig. 20, druse from mature parenchyma cell showing stalk, sheath, and air spaces at cell corners (dark shadows); fig. 21, sheath left after solution of crystal in similar cell.

The number of cells in any vertical file ranges from three or four to twelve or more, and in general the total length of the file may equal the length of the adjacent fiber. The walls of the septate elements thicken and may lignify unilaterally, either partially or completely.

Like druses, solitary crystals appear first as simple units, cubical or rhombohedral, 1–3 μ in size when first detected microscopically. There is no evidence that solitary crystals must necessarily arise by recrystallization of druses (12), although an occasional minute druse may occur in this tissue. In position the solitary crystals are similar to druse crystals, central in isodiametric cells, evenly

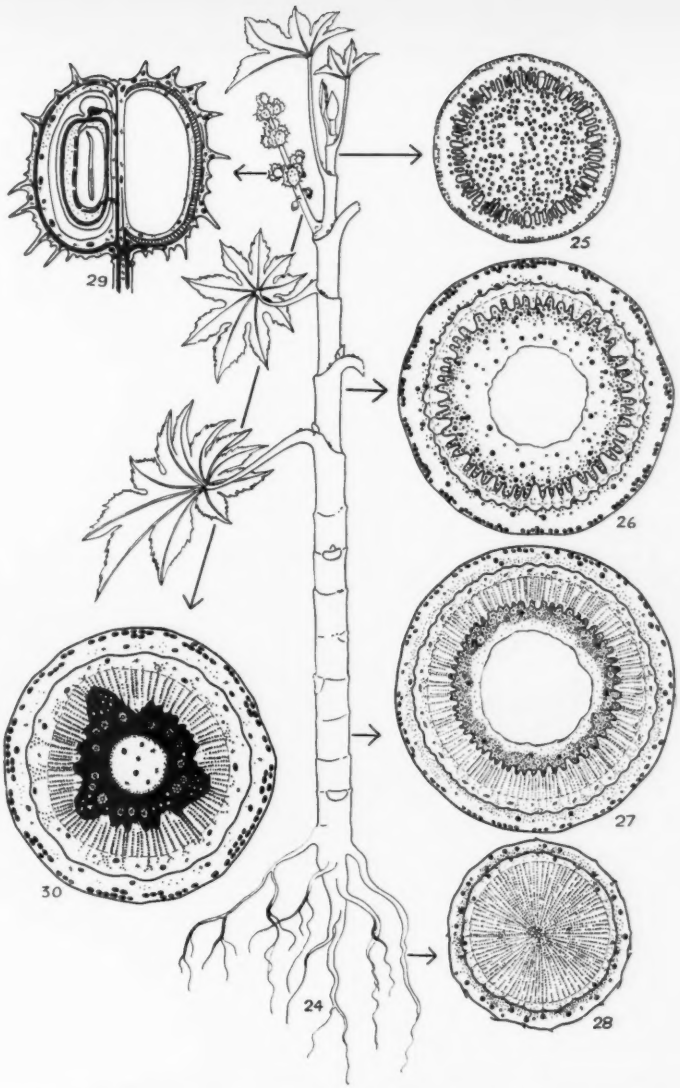


FIGS. 22, 23.—Solitary crystals in secondary phloem: fig. 22, longisection; fig. 23, transection

spaced in elongated cells. Crystal sheaths of undetermined composition remain after solution of these small crystals. As in the case of the druse, the mature solitary crystal is inclosed within a heavy cellulose sheath, practically fused to the cell wall or visibly attached by one or more very short stalks. The exact time at which sheath formation begins remains so far undetermined.

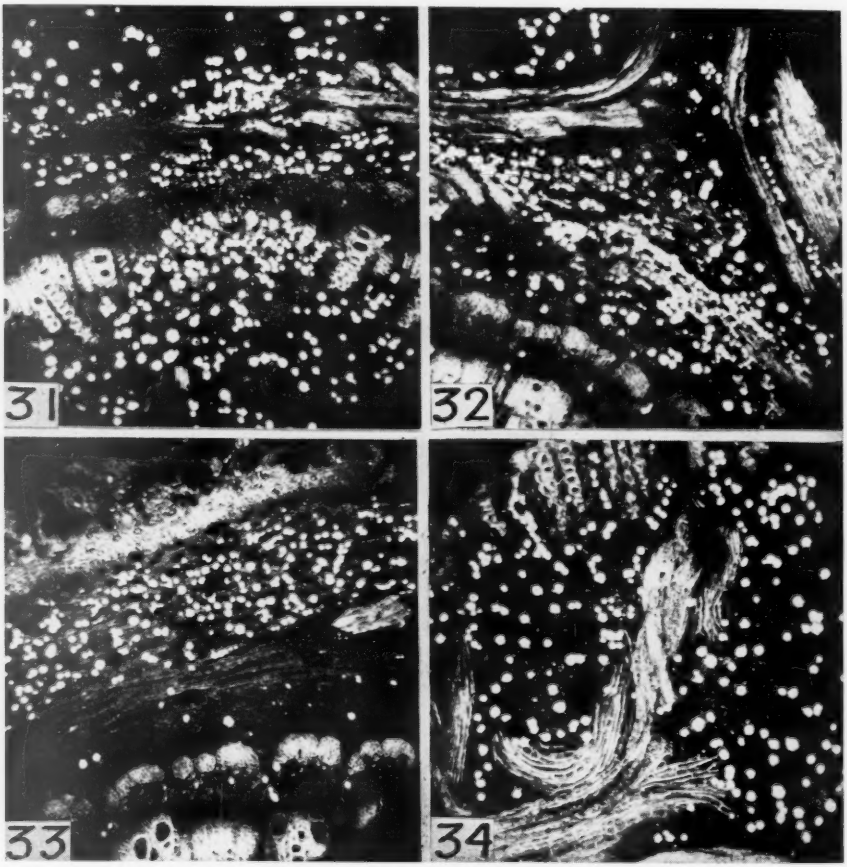
CRYSTAL DISTRIBUTION IN MATURE PLANT

Since crystal distribution is considered in relation to tissue differentiation, it is necessary to review briefly the anatomy of the plant as a whole (figs. 24–30). The



FIGS. 24–30.—Distribution in mature plant of calcium oxalate (crosses), fatty substances (larger black circles and ellipses), and starch (stippling or solid black in pith of fig. 30): fig. 24, young fruiting plant, eight internodes within terminal bud; fig. 25, transection, eleventh internode, druses and fat idioblasts alternating spatially in pith; fig. 26, transection, fifteenth internode, starch beginning to appear in outer pith and rays; fig. 27, transection, leafless stem, starch completely filling rays, and in outer pith partially masking fat idioblasts and crystals; fig. 28, transection, root; starch is dominant reserve material throughout entire root system; fig. 29, longisection, green fruit; calcium oxalate, fat, and starch relatively sparse; fig. 30, transection, fruiting branch as seed ripening begins; pith and rays completely packed with starch (solid black and stippled, respectively). All drawings diagrammatic.

main shoot of a typical vigorous plant of one season's growth may reach a height of 1–2 m. or more and a diameter of 4–6 cm. at the junction of shoot and root at ground level.



FIGS. 31–34.—Distribution of druses and fat idioblasts in region of node (blackened with osmic acid; semi-dark field): fig. 31, transection below leaf gap; fig. 32, leaf base and leaf traces; fig. 33, cortical region; fig. 34, part of vascular nodal septum.

Although the phyllotaxy is 2/5, only rarely 3/8, the first three regions of the stem each include eight internodes, 1–8, 9–16, and 17–24 (**15**). The leaves reach their maximum size about the level of the twenty-fourth node, and leaf fall may begin at the twenty-fifth. Lenticel and cork formation are evident at varying distances below this level. Flowering may begin when the plant is about 1 m.

high, and the fruits develop rapidly. The tap root is well developed and bears numerous secondary roots. Tissue differentiation is considered in the following sections in relation to crystal distribution.

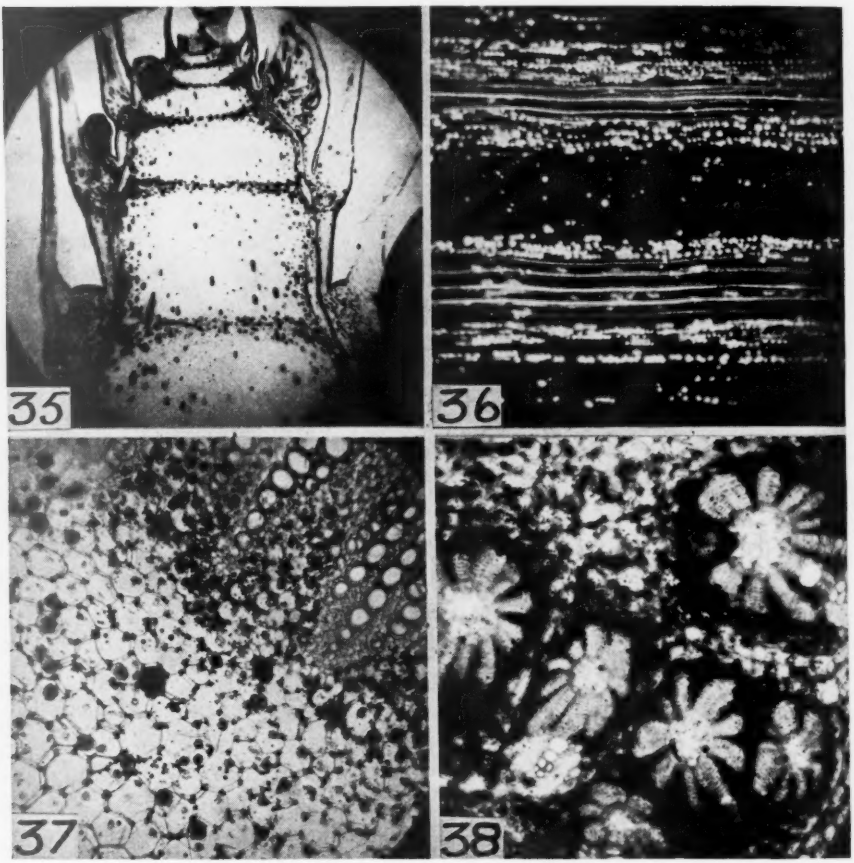
APICAL BUD (fig. 35).—Within the bud sheath, cell division is active in meristematic and in vacuolating dividing cells (**14**). Vascular differentiation is evident in the spiral elements at the base of the third left primordium and thence downward (**18**). Phloem keeps pace with xylem development. Anthocyanin, chlorophyll, tannin, sugar, and protein (Millon's reagent) are present in this region in small amounts, but the outstanding ergastic material is unsaturated fat. Fat idioblasts immediately reduce osmic acid. They occur sporadically but in gradually increasing amounts in the epidermis, pith, and other differentiating parenchyma cells and are particularly abundant at the level of the nodes. In pith and in parenchyma in general they may occur either as isolated cells or as vertical files two to ten or more cells in height, and one or occasionally two or more cells in width. Careful control of the osmic reaction shows the fatty substances in an extremely fine state of dispersion throughout the entire cytoplasm. This fine dispersion, of course, is the cause of the complete blackening of the cell contents.

The first traces of calcium oxalate in the apical region are presumably minute points of light visible in the differentiating parenchyma tissues under dark-field illumination or with crossed nicols. At the level of the third node (the region of the earliest vascular differentiation), minute druses 1–3 μ in diameter may be identified in the pith. In the succeeding internodes crystal idioblasts appear first as isolated cells which alternate in space with the fat idioblasts. They increase in number and, in the seventh and older internodes, vertical crystal files of varying length and width roughly parallel the fat-containing elements (figs. 12, 13).

In the pith, both fat and crystal idioblasts are usually surrounded by a ring of normal parenchyma cells. Those surrounding the fat cells contain some finely dispersed fatty substances and darken slightly with osmic acid. No such reaction occurs around the druse-containing cells. The two types of idioblasts therefore appear as metabolic centers of calcium oxalate and fat formation, respectively, and between them partition the territory of the pith parenchyma.

PRIMARY STEM (figs. 25, 39, 41).—In the primary stem the following tissues are clearly defined: epidermis, hypodermis with occasional large tannin sacs, cortical chlorenchyma and collenchyma, starch sheath, pericyclic parenchyma and fibers, vascular tissues, rays, and pith. The pith, except at the nodes, is hollow from the tenth internode onward. The persistent nodal septa consist of a complex network of vascular tissue in a ground tissue of living parenchyma. Differentiation of the vascular tissue of the node keeps pace with that of the stem (**16**). Chloroplasts are large and numerous in cortical chlorenchyma and in certain cells of the phloem

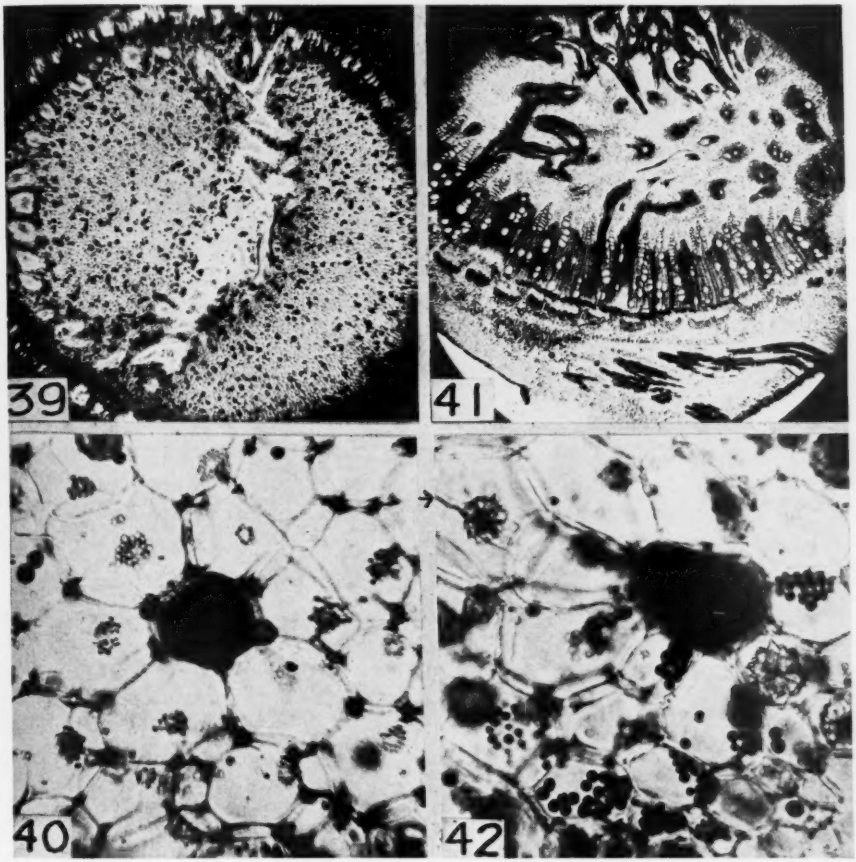
rays. A few smaller chloroplasts appear in the collenchyma, in the pith, and in the remaining parenchyma of the vascular system.



FIGS. 35–38.—Sections of stem apex, leaf vein, secondary region of stem, and inflorescence axis: fig. 35, longisection of stem apex showing fat idioblast distribution; faint grey dots are calcium oxalate druses; fig. 36, longisection of leaf vein showing druses in adjacent parenchyma (dark field); fig. 37, transection of seventeenth internode; starch grains beginning to appear in rays and outer pith; fig. 38, transection of fruiting axis; parenchyma cells adjacent to medullary bundles packed with starch.

The pattern of distribution of ergastic substances as outlined in the region of the apical bud is now clearly defined. Fat and calcium oxalate are the dominant metabolic products. In addition the following other substances are present in lesser amounts: (*a*) Transitory starch grains, minute in size, appear in the photo-

synthetic tissues. (*b*) Anthocyanin occurs sporadically in epidermal and subepidermal cells. (*c*) Tannin is present in the same tissues and is also found more or



FIGS. 39–42.—Fig. 39, eleventh node showing vascular network, fat idioblasts, druses faintly grey; fig. 41, older node, vascular network after treatment with phloroglucin and HCl. Figs. 40, 42, sections of pith; fat idioblast (blackened by osmic acid) surrounded by starch-containing cells; note stalked druse in fig. 42.

less sparingly in the pith; specialized tannin sacs of considerable size occur either immediately beneath the epidermis or deeper within the cortical chlorenchyma. (*d*) Fehling's solution tests indicate the general distribution of sugar, with an apparent maximum in the region of leaf base and nodal septum.

Fat idioblasts occur in the epidermis and throughout the parenchyma tissues and are very abundant at the level of the nodes and the leaf bases. Druses appear in parenchyma and also reach their greatest concentration in the nodal region. In the pith the spatial alternation of calcium oxalate druses and fat idioblasts is generally maintained (figs. 12, 13). In this region, therefore, it appears that the maximum distribution of both fat and calcium oxalate occurs at the level of leaf base and node and coincides with the expected and apparent maximum of sugar (figs. 29–32).

REGION OF SECONDARY THICKENING (figs. 26, 27, 31–34).—The spiral vessels of the primary system are succeeded by pitted vessels and fibers. Secondary phloem consists of alternate bands of functional sieve tubes with adjacent companion cells and parenchyma, and of phloem fibers with adjacent crystal idioblasts (figs. 22, 23). As in *Nicotiana* (4), secondary sieve tubes possess steeply oblique sieve plates. "Sieve-tube" starch is invariably present. Phloem fibers arise from fusiform elements, thicken basally as to walls by the addition of successive lamellae, and appear to maintain throughout their development a plastic growing tip as in *Boehmeria* (1). Adjacent to the fibers lie the solitary crystal idioblasts (3) and near them phloem parenchyma cells with large chloroplasts.

As regards ergastic substances, calcium oxalate and fat still remain dominant and reach their maximum density at the level of leaf base and node, but in starch distribution a marked change is apparent. Hitherto starch has appeared only as minute transitory granules within the chloroplasts. At this level measurable grains of storage starch begin to fill up the cells of the outer pith and xylem rays and are also evident to some extent in the cortical chlorenchyma (figs. 37, 40, 42).

LEAFLESS MATURE STEM (fig. 27).—Secondary thickening continues normally in the vascular cylinder, and on the surface of the stem lenticels become conspicuous. A cork cambium, epidermal or subepidermal in origin, appears and sooner or later becomes continuous.

In the ergastic substances of this region a striking change is at once apparent. Starch, hitherto a minor product, is now dominant. The rays and practically all the parenchyma tissues are completely filled with starch grains. Calcium oxalate druses and fat cells are now masked more or less by the surrounding starch-filled cells. Here and there empty crystal sheaths indicate the disappearance of druses, and starch grains develop within the former crystal idioblast. This disappearance of crystals, observed somewhat infrequently in the stem, is very common—as will appear later—in the inflorescence and fruiting axis.

Druse cells, fat idioblasts, and normal pith parenchyma cells do not grow at the same rate. In the youngest internodes the scattered fat idioblasts increase in volume more rapidly than in the surrounding parenchyma cells (table 1). They expand against the latter and maintain contact with them, so that no intercellu-

lar spaces develop at their surface. Later, beyond the bud sheath, the parenchyma cells make up for lost time, grow rapidly, equal and eventually surpass the fat cells in volume. Intercellular spaces now develop between parenchyma cells and idioblasts. In contrast to this, the druse idioblasts of the fourth internode are equal in size to the pith parenchyma cells, but they do not keep pace with the growth of the latter. Intercellular spaces consequently are apparent practically from the time that the druse idioblasts first appear.

TABLE 1

COMPARISON OF MAXIMUM SIZE OF NORMAL PARENCHYMA AND DRUSE AND FAT IDIOBLASTS IN PITH OF VARIOUS INTERNODES

No. of internode	"Average" diameter of cell (in microns)*		
	Normal parenchyma	Fat idioblast	Druse idioblast
1 (apex)	8	8	.....
4	21	27	21
8	38	50	34
10	57	52	38
12	99	70	43
14	133	85	66
18	228	152	76
28	228	114*	57

* Cells of pith may be isodiametric or radially elongated. In the latter case, "average" diameter is taken as the mean of the two dimensions of the cell apparent in longisection. Typical largest cells in internodes were measured.

All cells, fat idioblasts, druse cells, and normal pith parenchyma continue to increase in size until about the level of the fifteenth internode. The rate of growth of fat cells thus exceeds that of pith parenchyma in the first eight internodes within the bud sheath. The growth of druse idioblasts, in contrast to this, lags behind that of the parenchyma cell from the time of the first appearance. Shrinkage of fat idioblasts appears occasionally in the oldest tissues (table 1).

Leaf (fig. 36).—In the leaf veins calcium oxalate crystals are evident in practically all stages of development. In the caducous stipular sheath of the terminal bud, druses occur adjacent to the sheath traces. They appear at the leaf base of the third and succeeding leaves. As soon as intercellular spaces appear generally throughout the lamina, druses appear sparsely in the mesophyll itself and abundantly in the parenchyma which surrounds the vascular bundles of the projecting veins. The center of the leaf consists of a network of converging veins, similar in its complexity to the nodal vascular septum. As in the latter, calcium oxalate is again abundant.

Throughout the petiole tissues the distribution of druses is similar to that in the stem, the greatest number occurring in the central pith and occasional crystals elsewhere in the parenchyma. The number increases toward the petiole base.

Just as in the stem, fatty substances in the petiole and leaf base parallel the crystal distribution. They appear intermittently in epidermis and subepidermis, in cortex and vascular parenchyma. In the pith they alternate in space with crystal idioblasts and increase markedly in abundance toward the leaf base.

Within the lamina the maximum distribution of fat idioblasts occurs in the region of maximum concentration of calcium oxalate, namely, in the network of convergent veins at the leaf center and along the projecting veins themselves. In the latter, fatty substances appear in the pith of the vascular cylinder, in xylem and phloem parenchyma, and above and below the vascular strands. Fat is also present in certain epidermal and mesophyll elements.

In the leaf bases of older leaves, starch accumulation begins and keeps pace with the deposition in the older internodes. The same masking of crystals by starch grains may occur sooner or later, prior to leaf fall.

INFLORESCENCE AND FRUITING AXIS (figs. 30, 38).—The inflorescence axis bears staminate and pistillate flowers protected in the bud by scale leaves only. It resembles the vegetative shoot in essential structure but differs in certain characters which are obvious during the later fruiting stages: (1) Since it lacks vegetative leaves the axis is devoid of the complex vascular network of the vegetative nodes. (2) The inflorescence axis does not increase rapidly in diameter as does the main shoot. As a result the pith remains a solid continuous cylinder, usually with a central core of nonliving air-filled cells. Lignification of walls is common throughout the entire tissue. (3) The vascular supply of the flower pedicels appears as a group of medullary bundles traversing the outer pith. The bundles are concentric in type; xylem vessels and a heavy sheath of fibers surround a central strand of phloem. In the latter, sieve tubes are at once conspicuous by their starch granule content.

The change from fat to starch metabolism, evident during the secondary thickening of the vegetative stem, is paralleled in the growth of the fruiting branch. The axis differs in ergastic materials during various stages of growth.

In the early stages of development of the unopened buds and inflorescence axes the ergastic substances are similar in distribution and amount to those of the young vegetative shoot. Thus fatty substances are evident in epidermis, subepidermis, and pith, and also sparsely distributed in cortical and vascular parenchyma. Calcium oxalate is notable in the pith, and occasional crystals may appear in other parenchyma cells. Conspicuous starch grains delimit the starch sheath. Smaller granules of phloem starch are present in the sieve tubes, while minute granules may also be detected in the cortical chlorenchyma.

Active accumulation of starch begins with the setting of fruit. (The green fruit measures approximately 2×2 cm., including the spines.) Here, in addition to an increasing number of endodermal starch grains and sieve-tube granules, conspicuous grains are evident throughout the outer pith, particularly around the medullary bundles. Similar grains are also present in the xylem rays. The air-filled central cells, lignified and dead, naturally remain devoid of starch, but the fat and calcium contents of some cells still remain *in situ*. In the chlorenchyma the starch grains are now abundant. By the time the fruit is ripe the outer pith and the ray cells are solidly packed with starch grains. Sieve-tube starch is still evident, and many cortical phloem parenchyma cells are also heavily loaded. During this period the majority of calcium oxalate druses in the pith disappear and the crystal idioblasts become filled with starch grains (**2**). To begin with, two or three starch grains appear within the druse cell. The crystals now begin to break down and the starch grains increase in number. Occasionally empty crystal sheaths may be detected, surrounded by a "corona" of starch grains. Increasing starch accumulation naturally ends by obscuring all traces of the original crystal sheath.

In the developing fruit itself and in the seed, calcium oxalate, fat, and starch appear more or less abundantly at different stages of growth. The wall of the spiny, 3-locular dehiscent fruit consists of photosynthetic and vascular tissues and a solid layer of sclerenchyma fibers which eventually harden the capsule wall (fig. 29). In developing fruits (3–5 mm. or more in length) both calcium oxalate and fatty substances are rather abundant. Their concentration, as in other parts of the plant, is at a maximum in the region of vascular tissue, the carpel traces and the vascular network of the fruit base. Starch may appear in older fruits but is relatively sparse.

In the seed, in contrast to the fruit, calcium oxalate is rarely observed at any stage of development (**6**), and fatty substances similar to those of the vegetative tissues are likewise sparse. In the young ovule the inner integument is filled with starch. This disappears as the integuments begin to harden into the testa, and as—at the same time—protein and fat accumulate in the endosperm around the developing embryo. Endosperm fat differs from vegetative fat since it does not immediately reduce osmic acid. Occasional druses, very small in size, may occur near the micropyle, and minute crystals—detectable only under dark-field or polarized light—appear in the adherent nucellus. No traces of such crystals are observed at any time in the endosperm.

Root (fig. 28).—The young root is generally tetrach in structure (**20**), and the tissues, except for collenchyma and chlorenchyma, are essentially similar to those of the stem. Roots 0.05–5 cm. in diameter were examined in seedlings and in full grown plants. In the youngest roots (0.05–0.15 cm.) calcium oxalate is not observed. By the time the diameter measures 0.5 cm., occasional druses appear in

the rays and in the cortex near the bases of branch roots. Druses remain infrequent, even in the largest roots. On the other hand, solitary crystals appear adjacent to phloem fibers as soon as secondary thickening begins and continue to develop in the older roots.

The dominant ergastic substance of the root is starch. In roots 0.1 cm. in diameter numerous grains are already apparent in rays and cortex. Accumulation continues, and in the older roots the ray cells are closely packed with starch. A certain amount is also present in phloem parenchyma and periderm. Fat distribution, in contrast to the condition in the stem, is sparse. A few fat idioblasts appear only in cortical and vascular parenchyma cells. Tannin sacs similar in size to those in the stem may occur in the cortex, and a few tannin-containing parenchyma cells are evident here and there.

Crystal distribution in seedling

Seedlings in different stages of growth were examined. In the hypocotylary arch stage of development (length 2–4 cm.) the cotyledons are still inclosed within the endosperm. When the cotyledons emerge above the now straightened axis (length 5 cm.) the hypocotyl shows traces of chlorophyll and anthocyanin. As the foliage leaves expand, the full length of the hypocotyl is reached (about 7 cm.).

Calcium oxalate is only barely evident at any stage of development (figs. 14–17). While the cotyledons are below ground no calcium oxalate is observed in cotyledons, hypocotyl, root, or endosperm. As the cotyledons become green, a few druses appear at the cotyledonary node. In the older seedlings the druses increase markedly in number at the cotyledonary node and in the cotyledonary petioles. A few appear at the nodes of the growing vegetative leaves, but they are not yet obvious in the lamina.

Etiolated shoots.—In order to compare calcium oxalate distribution in etiolated and normal shoots, plants approximately 1 m. high—and therefore with a well-developed root system and ample reserve materials—were cut off about 20 cm. above the ground. They were then protected from the light by ventilated metal containers. The temperature remained reasonably well controlled, and—even on the hottest days in mid-August—did not exceed outside temperature by more than 3° F. Other plants, pruned in the same way, were allowed to grow in the open as controls.

As a result of the severe pruning, numerous lateral shoots develop from the basal nodes. Within 6 weeks, shoots on the control plants bear four to six leaves, and removal of the screens from the darkened plants shows a similar growth of etiolated shoots. The latter are devoid of chlorophyll, the laminae are butter yellow in color and the petioles pure white. Apart from color, control and etiolated shoots differ in the usual way in area of leaves and in length of petioles and internodes (table 2).

The distribution of calcium oxalate and other ergastic substances in the green shoots does not differ from that described for the main vegetative axis. On the other hand, in etiolated shoots, while the general plan of distribution remains the same, the relative amounts of materials vary considerably. Calcium oxalate druses are apparent as usual in parenchyma of pith, cortex, vascular cylinder, nodal septa, and leaf bases, but are relatively scarce. In the leaf veins and network of veins at the junction of petiole and lamina (in contrast to the crowded condition of the normal leaf) only a very few crystals are detected. Other ergastic materials,

TABLE 2

COMPARISON OF GREEN AND ETIOLATED SHOOTS

Type	Total length (cm.) of typical 5-internode shoot with terminal bud and leaves	Length (cm.) of terminal bud (TB) and succeeding internodes (A–E)						Internodes					
		TB	A	B	C	D	E		A	B	C	D	E
Green	3.6	0.5	0.3	0.5	0.8	0.8	0.7	Leaf size*	2.5×3	6×7	7×9.5	6×7.5	
								Petiole length	1	3.5	5.5	6	
Etiolated	6.1	0.7	0.4	1.9	2.5	0.5	0.8	Leaf size	1.6×2 (still folded)	2.5×3.5	Wilted	2.5×3.2	4×4.5
								Petiole length	0.2	2.5	6.5	7.5	3.2

* Length of midrib and greatest width.

such as fatty substances and tannin, do not appear to be significantly reduced in amount. In the absence of light, the starch grains of photosynthesis are naturally lacking in etiolated tissues, but the endodermis is clearly marked by starch grain content. The endodermis of the etiolated shoot is further delimited in the older internodes by the presence of typical Casparian strips, as described by PRIESTLEY (13) in *Vicia faba*. Starch grains, small but numerous, are present also in the guard cells of the stomata.

ETIOLATED SEEDLINGS (fig. 17).—Beneath the light screens, by the darkened plants, some stray seeds happened to germinate and reach the straight hypocotyl stage before dying down. These afford an interesting contrast to normal seedlings in height, color, and relative development of organs. A typical slender hypocotyl, perfectly white in color, may measure 33 cm. (more than four times the normal length). The cotyledons, butter yellow in color, remain reduced in area, actually

about one-third their normal size. The plumule remains unexpanded, while the root system appears to be normal in extent.

Sectons cut at different levels indicate a scarcity of all ergastic materials. At the cotyledonary node and in the cotyledonary petioles one or two small druses are seen, but none appear in the cotyledons. Calcium oxalate is practically absent in the hypocotyl and root.

Fatty substances occur in the cotyledons and at the cotyledonary node. They occur sporadically in the epidermis of the hypocotyl and in a few cells of the transition region. Elsewhere they are scarce. Starch is extremely limited in amount. It appears in the guard cells of the cotyledons and in the endodermis in the upper hypocotyl. Elsewhere, except for small sieve-tube starch grains, it is absent.

Discussion

That crystal formation is dependent on the presence of calcium is apparent from the work of THODAY (**22**), and more recently OLSEN (**11**) has demonstrated a definite quantitative relation between calcium absorbed and oxalic acid produced and precipitated. THODAY, in the apical meristem of the succulent *Kleinia*, found a scarcity of calcium and complete absence of oxalate crystals. Both calcium and crystals appear in increasing amounts in the older parts of the plant, toward the plant base, and reach their maximum in the vascular network of the nodes. In *Ricinus* the apical meristem is likewise completely free of crystals, and their maximum distribution occurs along leaf veins and in the vascular networks of leaf centers, leaf bases, and nodes. It may be expected that microchemical analysis will indicate a lower and a higher calcium content in apical meristem and in differentiated regions, respectively.

Crystal and fat idioblasts are obvious first in the youngest internodes, in the pith. This tissue is derived from vacuolating dividing cells and is apparently homogeneous. Conditions of growth are presumably similar for all cells. It may therefore be deduced that the fate of a cell, whether destined to become a fat idioblast, a crystal idioblast, or a typical parenchyma cell, is already determined within the apical meristem. The intracellular factors of the specific anlagen emerge during meristematic division and dominate the later extra-cellular environment.

Comparison of cell sizes in the pith (of fat and druse idioblasts) indicates, in the more rapid growth of the former, an apparent conflict between the two types of metabolism, fat accumulation and calcium oxalate deposition. Within the tissue the centers of fat metabolism are more or less evenly spaced and consist of the central idioblasts—conspicuous by the immediate reduction of osmic acid—and the surrounding cells in which fatty substances are present in lesser amount.

The fatty cell groups are the first to expand and become established as metabolic units. Their continued activity implies a diversion and monopoly of materials necessary for growth, the result of which may be the dwarfing of the marginal crystal idioblasts.

The actual density of crystal formation appears to depend on the amount of ergastic substances present (**9**). Thus etiolated shoots and seedlings, growing at the expense of food stored in root and endosperm, respectively, are relatively poorly supplied with the sugar, etc., necessary for growth, and crystal formation is therefore slight.

In the older stem, starch metabolism—the deposition of storage starch—becomes dominant. Here, far from increasing in number at the nodes or elsewhere, calcium oxalate crystals may dissolve and disappear, and the starch-filled crystal idioblasts can be recognized only by their relative size. Quantitative macrochemical analyses are necessary to give an accurate estimate of the actual disappearance of calcium oxalate. The root, during all but the primary stages of development, is a region of starch metabolism, and calcium oxalate crystals are practically entirely absent. Conditions incidental to starch deposition are therefore unfavorable for calcium oxalate deposition and may actually cause the disappearance of fully developed crystals.

Conditions of solitary crystal growth have been occasionally discussed in connection with conditions of wall thickening (**7**), either in the crystal idioblast itself or in the adjacent sclerenchyma element. Wall thickening per se can hardly be considered as a potent factor in crystal environment, since crystals are somewhat exceptional in typically thick-walled xylem tissue.

In view of the generally accepted concept of pH gradient in stem tissues, the distribution of crystal types appears at first sight significant, the more so since, as PFEIFFER (**12**) has shown, crystal growth can be controlled in vitro by varying pH and other conditions. Preliminary experiments with pH indicators (**21**) were attempted with *Ricinus* but proved unsatisfactory. In further work along this line, electrometric methods are to be preferred. In *Ricinus*, however, the fact that druses occur throughout the parenchyma in general, while solitary crystals are restricted to the "relatively alkaline" secondary phloem, appears to indicate a measure of specificity in pH conditions. This specificity is not maintained in other plants, however, since solitary crystals appear abundantly in the presumably "relatively acid" xylem fibers of *Prosopis*, the desert mesquite (**19**).

Summary

1. The development of calcium oxalate crystals and their distribution in the mature green plant, in etiolated shoots, in green and in etiolated seedlings, is examined in relation to tissue differentiation. Two types of crystals are present,

druses and solitary crystals. The former are distributed throughout parenchyma tissues in general; the latter are practically restricted to the secondary phloem, where they are deposited chiefly in vertical files of cells adjacent to the phloem fibers.

2. The development of a druse may be traced from a single crystal at the limit of visibility within a protoplasmic strand, through an aggregate or dendritic type of growth to a miniature druse, and finally to a mature crystal entirely filling the cell. Sheaths of undetermined composition may be detected around very small crystals about 3 μ in diameter. The sheaths of older druses consist of cellulose and are occasionally partly suberized. They are attached by stalks to the cell wall. Solitary crystals are also surrounded by cellulose sheaths.

3. Crystal idioblasts grow more slowly than adjacent parenchyma cells, and intercellular spaces appear around them in the youngest internodes. In contrast to this, fat idioblasts expand rapidly, maintain contact with surrounding cells, and intercellular spaces do not arise until later.

4. Crystals develop in the growing shoot about the level of the third node, and they rapidly increase in number in the differentiating primary stem. They reach their maximum concentration in the vascular networks of the nodes and leaf blade centers and along the leaf veins. Crystals are also distributed more or less regularly throughout the pith and are present also in the cortex and vascular parenchyma of the stem and of the petiole. They occur occasionally in the lamina and are frequent in the caducous stipular sheath. This greatest density of crystal deposition coincides with the maximum distribution of fat idioblasts and of sugar.

5. When secondary thickening is established in the stem, druses become masked by starch grain deposition in the pith and occasionally elsewhere. In many cases the crystals are dissolved and leave behind them the empty crystal sheath. At the same time starch accumulates and may completely fill the former crystal idioblast.

6. In contrast to druse behavior within the older stem, solitary crystals appear abundantly in vertical files of parenchyma cells adjacent to the phloem fibers in the secondary phloem.

7. In the root, except in the cortex near the origin of secondary roots, druses are practically absent. Solitary crystals, however, occur in the secondary phloem.

8. Druses are present in the pith of the young inflorescence axis approximately as in the stem. Since nodal vascular networks are absent, however, the crystals do not equal those of the shoot in number. As the seeds develop, starch deposition in the fruiting axis is extremely heavy; here, as in the older stem, druses may dissolve and disappear.

9. In the fruit wall, calcium oxalate crystals are not abundant at any stage of

development. In the seed, one or two small crystals may appear near the caruncle but do not occur elsewhere.

10. In the etiolated shoot, crystal distribution is similar to that in the green, but the number of crystals is markedly less.

11. In green seedlings, crystal development is slight, a few at the cotyledonary and first plumular nodes, rarely in the parenchyma of the hypocotyl, and occasionally in the cortex of the root.

12. In etiolated seedlings, crystals are practically absent. A few very small druses may be detected at the cotyledonary node. They are absent throughout the hypocotyl, and the plumular node does not develop.

13. Crystal distribution is discussed in relation to various factors, such as: calcium supply; tissue metabolism as indicated by the presence of fat, sugar, and starch; cell-wall thickening; hydrogen-ion concentration.

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CYTOLOGICAL AND TAXONOMIC STUDIES IN THE GENUS BRODIAEA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 530

MADELINE PALMER BURBANCK

(WITH TWENTY-NINE FIGURES)

Introduction

Brodiaea is the genus name commonly applied to a group of liliaceous plants which are widespread in the Pacific coast region of western North America. Certain plants native to South America have been included in the genus in the past, but it is probable that the unjointed pedicels and other characters of the South American plants justify their assignment to a different group. The genus *Brodiaea* ranges from British Columbia in the north to Lower California in the south, being especially common on the lower slopes of the Cascade and Sierra Nevada Mountains. Certain species extend as far eastward as Montana, Wyoming, Utah, Nevada, and Arizona. The plants produce seeds but in the majority of cases are more effectively propagated by offsets from the underground corms.

The twenty-five lots of plants, both species and named varieties of species, used in this study were all received under the genus name *Brodiaea*. Although this generic name had been most commonly applied to this group of plants, various subdivisions have been given in the past and again revived in recent publications by HOOVER (**4, 5, 6, 7**). In the first paper of this series, HOOVER summarizes the problem of synonomy in the genus, and it is not necessary to discuss the subject at length here. JEPSON (**8**) recognizes the genus *Brodiaea*, which he divides into three subgenera, Triteleia, Hookera, and Dichelostemma. ABRAMS (**1**) names six genera (*Calliprora*, *Hesperoscordum*, *Triteleia*, *Hookera*, *Dichelostemma*, and *Brevoortia*) to cover essentially the same plant material. More recently HOOVER's reports (**4, 5, 6, 7**), based on extensive field work in the western states, present morphological characters which he considers sufficiently distinct to justify a division of the plants into four genera. These are essentially the same groups as those recognized by JEPSON, with the addition of a new genus, *Triteleiopsis*. Under International Rules the reorganized groups become *Triteleia*, *Dichelostemma*, and *Brodiaea*. Since the plants used in this study bear out HOOVER's classification with respect to morphological characters and cytological observations, the generic and specific names he gives are, for the most part, used in this paper. Table 1 gives the synonomy. Species of *Brodiaea*, *Dichelostemma*, and *Triteleia* were studied, but plants of the monotypic genus *Triteleiopsis* were not available.

Brodiaea has previously been investigated cytologically by only three workers. In a list of plants possessing 10–12 unequal sized chromosomes, MÜLLER (10) in-

TABLE 1

SYNONOMY OF PLANT MATERIAL WITH EQUIVALENT REPORTED AND OBSERVED CHROMOSOME NUMBERS

As named by Hoover	As named when received (Purdy)	Chromosome numbers			
		Observed			Johansen
		2n	n		
Brodiaea	Brodiaea				
Coronariae					
B. elegans	B. grandiflora	32	16		.....
B. coronaria	B. grandiflora (Oregon)	42	21		2n=42 n=21
Stellares					
B. minor	B. purdyi	32	16		.....
B. minor var. nana	B. minor	12	6		2n=14
B. stellaris	B. stellaris	12	6	6*	2n=12
Appendiculatae					
B. californica	B. californica (Blue)	12	6		2n=10
B. californica	B. californica (Lilac Pink)	12	6		2n=10
Dichelostemma					
D. pulchellum	B. capitata	18	.....		2n=72
.....	B. capitata var. multiflora	18	.....		.....
D. congestum	B. pulchella	36	.....		2n=36
D. volubile	B. volubilis	18	9		2n=36
D. ida-maia	B. coccinea	48	.....		n=20(ca.)
Triteleia					
Eutriteleia					
T. grandiflora.....	B. douglasii	32	.....		.....
T. peduncularis	B. eastwoodii	14	.....		.....
.....	B. peduncularis	28	.....		.....
T. laxa	B. laxa	28(30)	14	14*	.....
T. laxa	B. laxa (Blue King)	42	.....		.....
T. laxa	B. candida	48	.....		.....
T. crocea	B. crocea	16	.....		.....
Calliprora					
T. ixioides var. scabra	B. ixioides var. splendens	10(11)	5(6)	5*	.....
T. ixioides var. analina	B. ixioides var. erecta	50	.....		.....
Hesperoscordum					
T. hyacinthina	B. lactea	28	.....		.....
T. hyacinthina	B. lactea (Oregon)	28	.....		.....
T. hendersoni	B. hendersoni	32		16*	.....
T. bridgesii	B. bridgesii	16	.....		.....

* Microspore counts.

cluded an unnamed species of *Triteleia*. JOHANSEN (9) worked with four species of *Hookera* (*Brodiaea*), four of *Dichelostemma*, and one of *Brevoortia* (*D. ida-maia*). The chromosome numbers he obtained are given in table 1, and in addition to those listed, he observed 30 somatic chromosomes in *D. multiflorum*. SMITH (12) reported the presence of chromosome rings and end-to-end attachment of more

than 2 chromosomes at microsporogenesis in *B. lactea*. Counts revealed about 21–24 pairs of chromosomes.

It is hoped that the present study will help to clarify relationships within the large group *Brodiaea*, through chromosome counts of approximately half the plants included in the genera *Brodiaea*, *Dichelostemma*, and *Triteleia*.

Material and methods

Plants of the twenty-four species and varieties used in this study were obtained from Mr. CARL PURDY, Ukiah, California, with the exception of corms of *B. coronaria*, which were received from Dr. FRANK H. SMITH, Corvallis, Oregon. Additional material of *B. lactea* (*T. hyacinthina*) was received from Dr. D. P. ROGERS of Corvallis, Oregon.

The corms were potted in sandy loam when received in the autumn. Four or five corms of each lot were kept in a cool greenhouse and used for root-tip material. Root tips were cut off as they emerged and were fixed in La Cour's 2 BE. The material was run up through the usual schedule of alcohols, chloroform and alcohol, and imbedded in paraffin. The root tips were cut transversely at 25 μ. The gentian violet-iodine method of staining, with—in some cases—a picric acid modification (**13**), was used for the root-tip sections. Immediately after being potted, the corms not used for root tips were placed in a coldframe, where they remained for several months during freezing weather. On being brought into a cool greenhouse many of the plants produced flower buds. Anther smears were made of appropriate stages of meiosis, both by the aceto-carmine method and by fixation of smeared anthers in Navashin's solution followed by staining with gentian violet-iodine. A few smears were also secured which showed the mitotic division of the microspore nucleus following meiosis.

As far as possible, some flower buds of each lot of corms were allowed to mature and the flowers used for a check on the identification of the plants. Unless stated to the contrary, fresh and pressed flowers were used for identification of all plant material. The descriptions given by ABRAMS (**1**), JEPSON (**8**), and HOOVER (**5, 6, 7**) were most frequently used, but references to the original descriptions were made occasionally.

A few selfings and cross pollinations were made with plants which bloomed profusely. Seeds were collected from five selfings and from one cross pollination. Several other pollinations produced negative results. No attempt has been made as yet to germinate the seeds obtained.

Unless otherwise stated, all drawings were made at table level with the aid of a camera lucida, using a 15 × compensating ocular and a 90 × apochromatic objective, n.a. 1.40, with a yellow-green filter. The magnification is approximately 2000 ×.

Identification of plant material and cytological observations

BRODIAEA

Within this genus HOOVER (5) recognizes four sections: Coronariae, Stellares, Appendiculatae, and Filifoliae. It is probable that two species of the Coronariae were employed in this study. The material received from PURDY under the name *B. grandiflora* was identified as *B. elegans* as described by HOOVER. The staminodia were acute, blunt, or irregularly three toothed, and always shorter than the stamens. The diploid chromosome number of this species is 32 (fig. 1), and 16 chromosomes (fig. 1*a*) were distinguishable in each early telophase I group. One chain of 4 chromosomes was seen at metaphase I (fig. 8). A few plants were selfed and two capsules of seeds obtained.

When the *B. grandiflora* material from Oregon bloomed at the same time as did the plants from PURDY, slight differences in gross morphology of the flowers could be detected. The tube of the Oregon flower was about 1 mm. longer than that of *B. elegans* and somewhat campanulate rather than funnelform; the segments were approximately the same length as those of *B. elegans* or slightly shorter and 1–2 mm. narrower; the staminodia were erect, not involute, tapered to an acute tip, and were the same length as the stamens; the anthers were 7 mm. in length as compared with 8–9 mm. in the material from PURDY. The leaves of the Oregon material were 2 mm. in diameter and were round or only slightly flattened, while those of *B. elegans* were 2–6 mm. wide and flattened. Chromosome counts of the Oregon material show a diploid number of 42 (fig. 2) and 21 chromosomes (fig. 2*a*) at metaphase II. Bridging was frequently observed in anaphase and telophase of meiosis I.

Although the Oregon material bloomed sparingly, the consistent flower differences, especially the relative lengths of stamens and staminodia and the distinctive chromosome number, suggest that the material is not *B. elegans*, which it resembles, but rather *B. coronaria* or a form of this species. The staminodia did not form a perfect "corona" but were not so far distant from the center of the flower as were those of the California material. A note from Dr. SMITH during the course of this investigation stated that the material which he sent as *B. glandiflora* had been identified as *B. coronaria* by Prof. HELEN M. GILKEY.

In the Stellares section, both root-tip and anther material of *B. minor* var. *nana* and *B. stellaris* showed that $2n = 12$ (figs. 3, 5) and $n = 6$ (figs. 3*a*, 5*a*). A metaphase figure of the microspore division was counted and verified the count of $n = 6$ in *B. stellaris* (fig. 5*b*).

Corms were received from PURDY under the name *B. purdyi*. JEPSON (8) does not mention this name, but both ABRAMS (1) and HOOVER (5) give *Hookera purdyi* and *Brodiaea purdyi* as synonyms for *Brodiaea* (*Hookera*) *minor*. Since certain

FIGS. 1–10.—Fig. 1, *B. elegans*, root-tip metaphase. Fig. 1*a*, same, early telophase I. Fig. 2, *B. coronaria*, root-tip metaphase. Fig. 2*a*, same, metaphase II. Fig. 3, *B. minor* var. *nana*, root-tip metaphase. Fig. 3*a*, same, metaphase I. Fig. 4, *B. purdyi*, root-tip metaphase. Fig. 4*a*, same, anaphase I. Fig. 5, *B. stellaris*, root-tip metaphase. Fig. 5*a*, same, metaphase I. Fig. 5*b*, same, microspore division. Fig. 6, *B. californica* (Blue), root-tip metaphase. Fig. 6*a*, same, metaphase I. Fig. 7, *B. californica* (Lilac Pink), root-tip metaphase. Fig. 7*a*, same, metaphase I. Figs. 8–10, associations of 4 chromosomes at metaphase I. Fig. 8, *B. elegans*. Fig. 9, *T. hyacinthina* (aceto-carmine smear). Fig. 10, *B. purdyi*.

floral parts of PURDY's *B. purdyi* were larger than the measurements given by HOOVER for *B. minor*, some doubt existed as to the correct names for the plants under investigation. The differences to be enumerated later leave no doubt that two distinct forms are involved. Since HOOVER separates *B. minor* var. *nana* from the species on the basis of smaller size without giving a complete set of measurements, the name is used here for the smaller plants. Although the other, larger plant is probably HOOVER's *B. minor*, the name *B. purdyi* is retained in this discussion as an indication that the plants did not conform in every respect to any description given by HOOVER.

In vegetative characters *B. purdyi*, in contrast to *B. minor* var. *nana*, showed more vigorous growth with longer and broader leaves and usually more than one scape from a single corm. Significant differences in length of plant parts are given in table 2. In addition, the segments of *B. purdyi* recurved soon after the flower

TABLE 2

MEASURED DIFFERENCES BETWEEN B. PURDYI AND B. MINOR VAR. NANA

PLANT PART	B. PURDYI	B. MINOR VAR. NANA
Scape	3–12 cm. (av. 8–9 cm.)	3–13 cm. (av. 6 cm.)
Tube	7–8 mm.	8–9 mm.
Segments	16–19 mm.	9–12 mm.
Staminodia	11 mm.	6–7 mm.
Anthers	6 mm.	4 mm.

opened, while those of *B. minor* var. *nana* did not. With increasing age the flowers of *B. purdyi* faded to a bluish violet, with a prominent, dark midvein, while those of *B. minor* var. *nana* darkened and shriveled.

Root-tip cells of *B. purdyi* contain 32 chromosomes (fig. 4). At meiosis I and II the reduced number of 16 (fig. 4*a*) can be observed. Occasionally there are associations of 3 or 4 chromosomes and one ring of 4 was observed (fig. 10). One plant set seed after being selfed.

The only other species of *Brodiaea* studied was *B. californica*. PURDY offers two color forms of this species, "Lilac Pink" and "Blue." Under greenhouse conditions these color differences were not always noticeable. The leaves of the Lilac Pink plants tended to be wider than those of the Blue. For both lots the mitotic chromosome number was 12 (figs. 6, 7) and the meiotic number 6 (figs. 6*a*, 7*a*). At both stages the chromosomes of *B. californica* Lilac Pink were slightly larger than those of the blue form. One of the corms received as *B. californica* Lilac Pink produced white flowers, but no irregularities of chromosome structure or behavior were observed at meiosis.

Dichelostemma

Of the six species and one variety listed by Hoover (**6**) in this genus, chromosome counts were made of four species and one variety. Neither *B. capitata* nor *B. capitata* var. *multiflora*, as received from Purdy, flowered in Chicago. According to synonomy, *B. capitata* is *D. pulchellum*, but a corresponding synonym for the variety "*multiflora*" was not found. In both plants $2n = 18$ (figs. 11, 12).

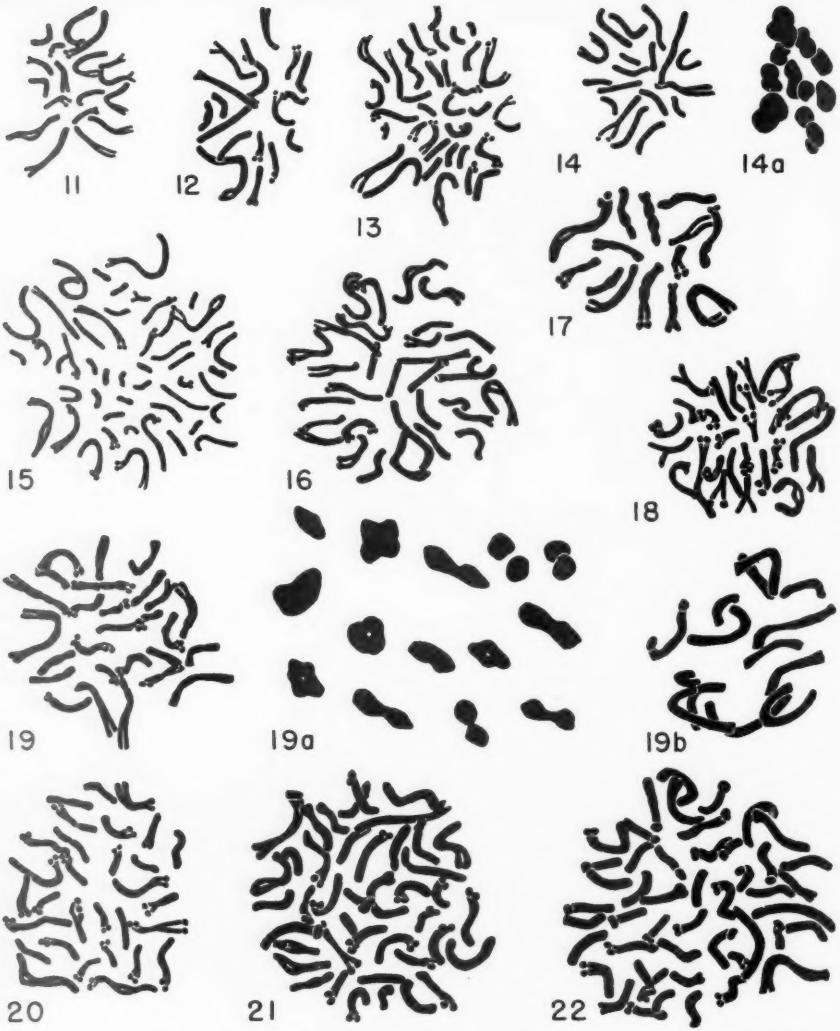
D. congestum has a diploid number of 36 (fig. 13). The few meiotic stages observed did not give a clear picture of the chromosomes, but the number of bivalents appeared to be approximately 18. *D. volubile* had a diploid number of 18 (fig. 14), and 9 bivalents (fig. 14*a*) were seen at metaphase I. No seeds were set following selfing. *D. ida-maia* has a diploid number of 48 (fig. 15). The large number of chromosomes made it difficult to distinguish individual bivalents at meiosis I, but at metaphase II approximately 24 chromosomes could be seen. One capsule of seed was collected following pollination of *D. volubile* with *D. ida-maia* pollen.

Triteleia

On the basis of the attachment and length of the anther filaments, Hoover (**7**) divides this genus into three sections, Eutriteleia, Calliprora, and Hesperoscordum. First among the plants in the section Eutriteleia, which have the stamens attached alternately in two rows on the perianth, is the type species of the genus *T. grandiflora*. The somatic chromosome number is 32 (fig. 16). Although a few flowers were produced which made it possible to identify the plants, there was not sufficient material for meiotic counts, since the corms tended to split into smaller units rather than to send up flowering scapes.

The second species of the Eutriteleia section is *T. peduncularis*. Some confusion exists about two distinct plants which have been called by this name. According to Purdy (private correspondence with the writer), the plants which he has under the name *B. eastwoodii* have been identified by western taxonomists as *B. peduncularis*. The plants which he carries as *B. peduncularis* are then without a name. This matter will be discussed in greater detail later. Since the plants did not flower, Purdy's determination of two morphologically distinct plants is accepted, and according to Hoover's nomenclature, *B. eastwoodii* is *T. peduncularis*, and *B. peduncularis* is unnamed. Examination of root-tip material reveals that for *T. peduncularis* $2n = 14$ (fig. 17), and in the unnamed species $2n = 28$ (fig. 18). The leaves of *T. peduncularis* were unique in that the midrib region was triangularly thickened. Between the acute angle of the keel and the upper surface of the leaf there was a space, slightly pithy, which in cross section measured about 2 mm. on each of the three sides.

According to Hoover (private correspondence and **7**), *T. laxa*, as he recognizes it, contains several forms, but no clear-cut separation can be made on the basis of



FIGS. 11–22.—Fig. 11, *D. pulchellum*, root-tip metaphase. Fig. 12, *D. pulchellum* var. *"multiflora,"* same. Fig. 13, *D. congestum*, same. Fig. 14, *D. volubile*, same. Fig. 14*a*, *D. volubile*, metaphase I. Fig. 15, *D. ida-maia*, root-tip metaphase. Fig. 16, *T. grandiflora*, same. Fig. 17, *T. peduncularis*, same. Fig. 18, *T.* unnamed, same. Fig. 19, *T. laxa*, same. Fig. 19*a*, *T. laxa*, metaphase I; bivalents drawn separately. Fig. 19*b*, same, microspore division. Fig. 20, same, root-tip metaphase with 2 extra chromosomes. Fig. 21, *T. laxa* Blue King, root-tip metaphase. Fig. 22, *T. candida*, same.

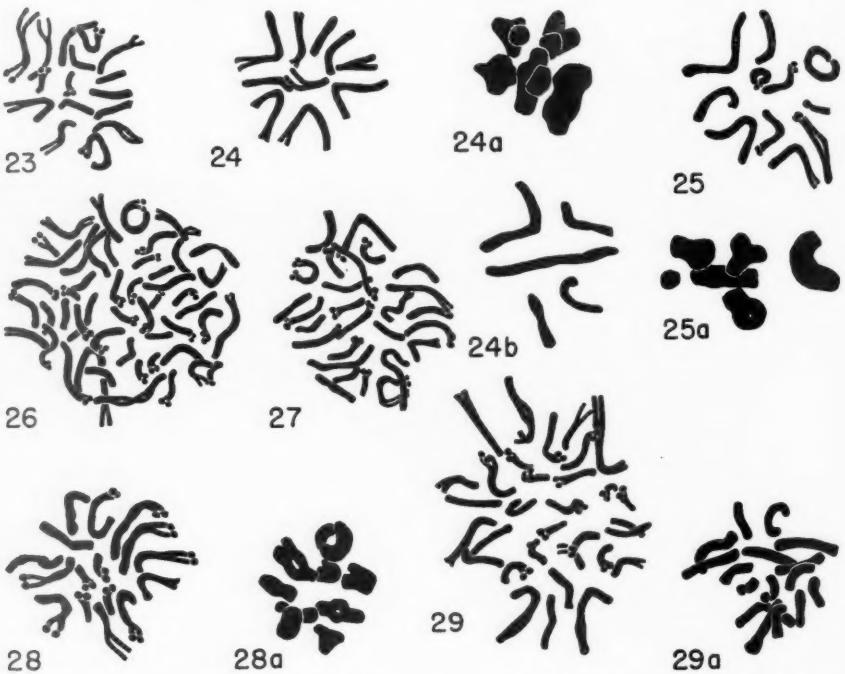
morphological characters. Material received from PURDY under the names *B. laxa*, *B. laxa* Blue King, and *B. candida* should all be called *T. laxa*, according to HOOVER. In this study, however, the species names as given by PURDY will be used with the genus name *Triteleia*, since the three plants are somewhat distinct taxonomically and cytologically. As grown in the greenhouse here, *T. laxa* Blue King usually had slightly broader leaves, longer scapes, and flowers of a deeper lavender-blue than did *T. laxa*. The material was rather variable, especially with regard to the horizontal or upright orientation of the flowers, the location of the pistils along the lower side of the flowers or in a central position, and blue or white coloration of the stamens and pollen. *T. laxa* shows $2n = 28$ (fig. 19) in root tip and $n = 14$ (fig. 19*a*) at metaphase of meiosis I and the microspore division (fig. 19*b*). One plant contained 30 chromosomes (fig. 20) in root-tip cells. The root-tip material of *T. laxa* Blue King shows $2n = 42$ (fig. 21). The massing of the large number made accurate counting of meiotic chromosomes impossible, but in a few cells two groups of approximately 21 chromosomes each could be seen at anaphase I. At diakinesis there appeared to be about 19–23 bivalents and 3–5 univalents, with very few chiasmata evident. The haploid number of 14 was seen at meiosis in at least one plant labeled *T. laxa* Blue King and in a microspore division figure from a similar plant where the expected number would be 21. Enlargement of the ovaries followed self-pollination in both *T. laxa* and *T. laxa* Blue King, but no seeds were produced.

The plants of *T. candida* differ from those of *T. laxa* in several respects. The flowers were produced 1–2 months earlier than those of *T. laxa;* they were slightly larger, and the leaves were wider and a more yellow green, with a succulent appearance. This was not caused by some local difference in microclimate because the plants were all treated in the same way, and the differences were apparent both winters that the plants were grown. The morphological characters of *T. candida* flowers were essentially the same as those of *T. laxa*, except for the smaller size of the anthers. PURDY (private communication) maintains that *T. candida* and *T. laxa* can be distinguished by the angled pedicels and horizontal flowers of *T. candida* and by the straight pedicels of *T. laxa*, which are never angled at the base of the tube. Since this information was received from PURDY after the *T. candida* flowers had ceased blooming, fresh material could not be examined, and pressed specimens were unsatisfactory on this point. As already stated, however, some flowers of both *T. laxa* and *T. laxa* Blue King formed an angle with the pedicel, so the distinction does not always hold true.

In *T. candida* $2n = 48$ (fig. 22). Again meiotic counts were difficult, but about 22–24 bivalents could be seen in several cases. Some of the configurations at metaphase I suggested multiple associations of chromosomes, but the material requires further study. Two capsules of seed were collected following self-pollination.

T. crocea, another member of the Eutriteleia section, had a somatic number of 16 (fig. 23). Since these plants did not bloom, no meiotic counts were possible, and PURDY's determination of the species was accepted.

The Calliprora section includes those plants in which the anther filaments are alternately long and short but all attached at the same level. The varieties of



FIGS. 23–29.—Fig. 23, *T. crocea*, root-tip metaphase. Fig. 24, *T. ixioides scabra*, same. Fig. 24*a*, *T. ixioides scabra*, metaphase I. Fig. 24*b*, same, microspore division (aceto-carmine smear). Fig. 25, same, root-tip metaphase with 1 extra chromosome. Fig. 25*a*, same, metaphase I with fragment. Fig. 26, *T. ixioides* var. *analina*, root-tip metaphase. Fig. 27, *T. hyacinthina*, same. Fig. 28, *T. bridgesii*, same. Fig. 28*a*, *T. bridgesii*, metaphase I. Fig. 29, *T. hendersoni*, root-tip metaphase. Fig. 29*a*, same, microspore division.

B. ixioides received from PURDY correspond with the two varieties of *T. ixioides* which HOOVER (**7**) places in this group, *T. ixioides* var. *scabra* and *T. ixioides* var. *analina*. Three of the four plants from which root tips were collected showed 10 somatic chromosomes (fig. 24) in *T. ixioides* var. *scabra*. The fourth plant had 11 chromosomes (fig. 25), which exhibited greater extremes in length than did those of the "normal" complement of 10. Meiotic counts from two out of about twelve plants examined revealed an extra chromosome or chromosome fragment at meta-

phase I (fig. 25*a*) in addition to the normal five pairs (fig. 24*a*) characteristic of the majority of plants. The extra chromatic material was in the form of a small circular mass. It was never seen associated with a bivalent and frequently was at some distance from the group of bivalents. If present in a single cell, the fragment was present in all cells on the slide in which individual chromosomes or bivalents were distinguishable. At anaphase I and telophase I the fragment appeared capable of going undisturbed to one of the daughter nuclei. Frequently the fragment became isolated in the cytoplasm or remained between the two masses of cytoplasm which rounded up around each nucleus preliminary to the formation of a cell wall between the daughter cells. None of the material obtained showed the fate of the fragment in meiosis II. The reduced number of five chromosomes was observed in one microspore (fig. 24*b*). Two plants set seed following selfing.

T. ixioides var. *analina* has a somatic chromosome complement of 50 (fig. 26). Meiotic stages in which the chromosomes could be counted were not obtained. In a few cells there was a small circular mass of chromatin separate from the mass of metaphase I bivalents. At later stages there was very rarely a "fragment" associated with one or both of the masses of chromosomes at telophase I and metaphase II. Attempted crosses between the two varieties with *T. ixioides* var. *analina* as the pollen parent were unsuccessful.

Chromosome counts were made of three species included in the section Hesperoscordum, which contains plants with equal stamens. The material of *T. hyacinthina* from Oregon was identical with that obtained from PURDY. Both had a somatic number of 28 (fig. 27). The plants did not flower profusely, and the meiotic material suggests 14 bivalents at metaphase I and 14 in each chromosome group at metaphase II, but the determination could not be made positively. A ring of four chromosomes (fig. 9) was seen in two separate cells.

T. hendersoni has a diploid number of 32 (fig. 29). No meiotic material was obtained, but the reduced number of 16 was observed at metaphase of the microspore division (fig. 29*a*). In the absence of any mature flowers, the species determination of PURDY is accepted.

In *T. bridgesii* $2n = 16$ (fig. 28). Eight bivalents were observed at metaphase I (fig. 28*a*). Self-pollination resulted in enlarged ovaries, but no seeds were matured.

Discussion

The preceding observations indicate that the plants commonly known as Brodiaeas comprise a far from homogeneous group. Taxonomically three divisions can be recognized. Cytologically there might be postulated five groups, with basic numbers of 5, 6, 7, 8, and 9, since the diploid numbers of 10, 12, 14, 16, and 18 occur as well as multiples of these numbers. Grouped according to known chromosome numbers, the lines of the natural divisions as outlined by taxonomists would

be entirely obscured. A more fruitful—and in this case perfectly justifiable—approach to the problem is to show that each of the three genera, *Brodiaea*, *Dichelostemma*, and *Triteleia*, comprises a cytological as well as a taxonomic unit. It is unfortunate that none of the species studied have trabants or other means of ready identification of individual chromosomes. Even the centromere cannot always be located. Comparative lengths of chromosomes are helpful when cells can be found in which the chromosomes all lie in the same plane throughout their length.

Within the genus *Brodiaea*, as used in the limited sense, there are found three somatic numbers, 12, 32, and 42. Six, the gametic number of three of the six plants examined, may be considered the basic number. The similarity of the chromosomes of *B. minor* var. *nana*, *B. stellaris*, and *B. californica* is clearly seen in figures 3 and 5–7. In each plant the shortest chromosome has an approximately subterminal centromere and the longest chromosome a median constriction, indicating the position of the centromere. Likewise each of the other four chromosomes in *B. minor* var. *nana* has its counterpart in *B. stellaris* and *B. californica*. Although each chromosome of *B. californica* is larger than the similar one in the other two species, the straight progression from shortest to longest is relatively the same for all three species. This indicates a close relationship between the species.

Plants received as *B. purdyi*, $2n = 32$, may be derived from a hexaploid variety of a *B. minor* var. *nana* plant containing 36 chromosomes. A theory such as the "dislocation" hypothesis of NAVASHIN (11) could account for the change from 36 to 32 somatic chromosomes. The observation of meiotic irregularities in material of *B. purdyi* lends support to a theory which involves changes in chromosome number and morphology. It is impossible to recognize the 6 basic chromosomes and determine the degree of "ploidy" by a critical examination of the somatic or meiotic cells of *B. purdyi*, but the general range of length is similar to that found in the plants in which $2n = 12$.

The chromosome numbers of *B. elegans*, $2n = 32$, and *B. coronaria*, $2n = 42$, do not suggest a close relationship between these species and those of the Stellares and Appendiculatae, although such a relationship may have existed at one time. *B. coronaria* could be considered a heptaploid, but the number for *B. elegans* is not a multiple of 6. It is possible that polyploidy could have produced two plants with counts of 36 and 48 from plants in which $2n = 12$. Subsequent additions and deletions of whole chromosomes or parts could then have brought about the evolution of the present numbers of 32 and 42. The quadrivalent association observed in *B. elegans* indicates that a reciprocal translocation has occurred. Such a hypothesis of the origin of the species does not correlate with HOOVER's statement (5) that the plants included in the Coronariae are the most primitive from the

standpoint of morphology. The facts, however, that *B. elegans* and *B. coronaria* have been confused by many taxonomists and put under one species, as may even be the case for the plants here studied, and that intermediate forms have been reported near the northern and southern limits of the range of *B. elegans*, suggest that here may be a group of plants in which chromosome number can be of aid in species delimitation. The species may be in the process of evolution through the development of ecotypes and ecospecies. The fact that *B. elegans* set seed indicates that these plants have at least reached an equilibrium which allows normal sexual reproduction, if the seeds produced are viable. The frequent occurrence of bridging in *B. coronaria* suggests that this species has not attained a similar degree of stability. Duplication of a set of 6 chromosomes cannot be identified; but as in *B. purdyi*, the general range in length and width of the chromosomes is similar to that in the other species of the genus.

JOHANSEN (**9**) previously reported chromosome counts for four species of *Hookera*, which is synonymous with *Brodiaea* as used by HOOVER (**4**). His $2n = 12$ for *B. stellaris* agrees with the present findings. His drawing of a root-tip cell of *B. californica* suggests that perhaps 2 of the longest chromosomes may in reality be 4. Such an interpretation would then give a somatic count of 12 as reported here. Conversely, 2 of the chromosomes of *B. minor* as drawn by JOHANSEN would need to be joined to 2 others to agree with the present count of 12. Such corrections are suggested on the basis of very clear mitotic and meiotic figures. The haploid and diploid numbers of 21 and 42, respectively, reported by JOHANSEN for *Hookera coronaria*, suggest that the material tentatively named *B. coronaria* in this study is indeed that species. Sufficient meiotic material was not available for JOHANSEN to observe the presence or absence of irregularities in chromosome behavior in *B. coronaria*.

Dichelostemma is the most homogeneous genus with respect to chromosome number. *D. pulchellum*, a variety of this species, and *D. volubile* each have a somatic number of 18. Nine bivalents could be clearly distinguished at metaphase I in *D. volubile*. *D. congestum*, which has 36 chromosomes, is then a tetraploid or a hexaploid, depending upon the point of view. On the basis of these four plants alone, and bearing in mind that they are assigned to a separate genus, it would be supposed that the basic number is 9 and that *D. congestum* is a tetraploid. Added to this evidence is that of the production of seed by *D. pulchellum*, which would be more usual in a diploid than in a triploid. On the other hand, *D. ida-maia*, with a number of $2n = 48$, is also included in the genus. Forty-eight is a multiple of 6 but not of 9. It has been suggested by many that, because of the peculiar type of flower, *D. ida-maia* should be assigned to a separate genus. There are, however, certain morphological similarities to the members of the genus *Dichelostemma*, and if it is true that *D. venustum* is a hybrid of *D. volubile* and

D. ida-maia, the latter would be expected to belong to the same genus rather than to a separate one. In this work several flowers of *D. volubile* were pollinated with *D. ida-maia* pollen, and two somewhat shriveled seeds were produced. Whether or not they are capable of germination has not yet been determined.

A hypothetical solution of the relationships of the plants included in *Dichelostemma* would be that far back in the beginning of the *Brodiaea* complex, 6 was the basic number. By various means one group of plants may have evolved with 9 as a secondarily basic number. Paralleling the duplication of certain chromosomes to give the diploid number of 18, an octoploid with 48 chromosomes may have arisen which in certain respects had a genetic make-up similar to that of the $2n = 18$ plant. Thus gross morphological characters might be similar and chromosome complements be sufficiently alike to allow crossing. It would be of interest to know the chromosome number of *D. venustum*.

JOHANSEN (9) counted the chromosomes of five species in this genus, one of which, *D. multiflorum*, was not obtained for this study. The reported $2n$ number of 30 might be considered to strengthen the hypothesis of 6 as a basic number. The counts of three other species by JOHANSEN agree in the case of *D. congestum* but are multiples of 18 for *D. volubile* and *D. pulchellum* (table 1). Such variations suggest the existence of polyploid races within a species which are indistinguishable in general morphology. JOHANSEN counted about 20 haploid chromosomes in meiosis in the microsporocytes of *Brevoortia* (*D. ida-maia*) and reported that this may be a plant with oscillating chromosome numbers. Although there was some difficulty in counting the chromosomes of *D. ida-maia* for this study, it was attributed to the fact that several very small chromosomes were easily obscured by the larger ones rather than to an actual variation in number. JOHANSEN mentions this striking variation in size. *D. congestum* likewise has some very small chromosomes. These short chromosomes, and the fact that all the species included in *Dichelostemma* tend to have much smaller chromosome diameters than do those of the other genera, further justify the separation of this group into a distinct genus.

Cytologically the genus *Triteleia* is distinguished by the variation in chromosome numbers among the species and the high proportion of species and varieties with meiotic irregularities. In addition, *T. ixioides* var. *scabra* and *T. laxa* plants contained individuals with chromosome numbers higher than the number in the majority of plants. This aneuploid condition is similar to that found by BEAL (2) in the Mariposa section of *Calochortus*. Somatic chromosome numbers of *Triteleia* include 10 (11), 14, 16, 28 (30), 32, 42, 48, and 50. Three groups can be recognized: (*a*) those with 10 (11) and the decaploid 50; (*b*) those with a diploid number of 14, the tetraploids with 28, and the hexaploid with 42; (*c*) those with a diploid number of 16 and the tetraploids with 32. The species with a diploid number of 48 could be a hexaploid in the third group or belong to a distinct group of

which the basic number was 6. The material examined gave no cytological clue as to possible relationships. Table 1 shows that the sections set forth by HOOVER (7) and the preceding three groups do not include the same plants. Further cytological and taxonomic work on the genus is needed to reconcile these differences.

No basic number for *Triteleia* can be suggested on the basis of present findings. Multiples of 7 and 8 occur in equal numbers. The presence of rings of 4 chromosomes in *T. hyacinthina*, rings of 4 and 6 chromosomes reported by SMITH (12) for *T. hyacinthina* plants with probably twice the chromosome number reported here, fragments in the two varieties of *T. ixioides*, the paucity of chiasmata and the presence of univalents in the *T. laxa* Blue King material, and the early separation of the small chromosomes at meiosis in the *T. laxa* material, all suggest that chromosome changes have played and are continuing to play a significant role in the evolution of species of *Triteleia*. No wonder it is a difficult group taxonomically. It is even possible that 6 was again the original number and other numbers have been derived from a diploid plant with 12 chromosomes. The mechanism for such changes has been outlined by NAVASHIN (11) and applied by BEAL (2) in his discussion of the Mariposa section of *Calochortus*.

The foregoing discussion of cytological findings has demonstrated that chromosome counts may be used to substantiate the division of *Brodiaea* into three genera. Each group is characterized by a separate basic number or by the absence of such a number. It is realized that the evidence is not conclusive, that on the basis of chromosome number alone, *B. elegans* and *B. coronaria* could be assigned to the *Triteleia* group or even all the plants considered a part of a single genus complex, but combined cytological and taxonomic findings indicate that the division into three groups is justified.

While the chromosome counts agree with taxonomic classification in general, this study indicates the need for taxonomic reexamination of certain species. First in *Brodiaea*, chromosome counts would indicate that *B. nana* var. *minor* is the species ($2n=12$) and that the material received as *B. purdyi* ($2n=32$) is a variety derived from the plants with a lower count. More vigorous growth as noted in *B. purdyi* is frequently associated with polyploidy. The presence of a ring of 4 chromosomes and a chain of 3 at meiosis I suggests polyploidy and chromosome rearrangement, including reciprocal translocation. HOOVER, in a private communication to the writer, suggested that the material which PURDY carries as *B. minor* may be *B. minor* var. *nana*. He further stated that EASTWOOD, who originally described *B. purdyi*, has seen the type specimen of *B. minor* in England and finds the two identical.[1] It then remains to re-examine the question of whether

[1] It is also interesting to note that the *B. purdyi* material grown here corresponds more closely with EASTWOOD'S (3) original description of the species than with HOOVER'S (5) description of *B. minor*. EASTWOOD gives the staminodia length as 13 mm. in contrast to 7–10 mm. and further states that the perianth segments spread widely, recurve, and possess a dark midvein.

B. minor and *B. minor* var. *nana* are a species and a variety or whether they each deserve specific rank. The difference in chromosome number lends support to the latter view, as do also the differences in the flowers as given earlier in this paper. A knowledge of the chromosome numbers of the forms reported by HOOVER (5) which are intermediate between *B. minor* and *B. minor* var. *nana* might be critical for a solution of the problem.

Taxonomic and cytological findings seem to be in agreement within the genus *Dichelostemma*. This is not so completely true in *Triteleia*. Chromosome counts may be a definite aid in the solution of the status of *B. peduncularis* and *B. eastwoodii*. The latter has never been "adequately published under existing rules of nomenclature" (HOOVER, private communication), and for that reason is not listed anywhere as a synonym by him. In the following discussion, however, *B. peduncularis* and *B. eastwoodii* will be used for the sake of clarity to designate the plants received from PURDY under those names. The diploid number of *B. eastwoodii* is 14 and that of *B. peduncularis* is 28. This suggests that the latter is a tetraploid which has arisen from *B. eastwoodii*. The greater length of peduncle reported by PURDY would further substantiate the theory. The isolated and limited habitat, supposedly inaccessible to early botanists, in which *B. peduncularis* was found by PURDY, may indicate that it is of recent origin and has not yet extended its range. Whether it was found by earlier botanists is questionable. PURDY maintains that his *B. peduncularis* more nearly resembles the plant described by WATSON than does the plant for which the description was made, PURDY'S *B. eastwoodii*. In this he is supported by Mr. BAKER of Santa Rosa Junior College. Both EASTWOOD and HOOVER maintain that PURDY'S *B. eastwoodii* is the true and original *B. peduncularis* as described by WATSON. PURDY suggests that perhaps type localities in some of the older collections are inaccurate, and that his *B. peduncularis* might have been collected and described as such. Later collectors might then have collected PURDY'S *B. eastwoodii* from the incorrectly given type locality of *B. peduncularis* and assumed the material to be *B. peduncularis* without examining it critically. Whatever is decided concerning the names and taxonomic classification of the two plants, the chromosome numbers indicate that they are closely related and that "*B. peduncularis*" could be a tetraploid derived from "*B. eastwoodii*."[2]

Observations made upon the plants received from PURDY under the specific and varietal names *T. laxa*, *T. laxa* Blue King, and *T. candida* indicate that here, indeed, are two species and a variety or form of one of the species rather than a single species, *T. laxa*, as described by HOOVER. *T. laxa* has a haploid number of 14 and diploid number of 28. The observance of 30 somatic chromosomes in the

[2] Private communications from PURDY are the source of much of the information given in this paragraph.

roots of only one plant indicates that this number is the exception and not the rule. The presence of 42 somatic chromosomes in *T. laxa* Blue King suggests that this is a hexaploid form of the *T. laxa* material. As the plants flowered in the greenhouse, those of *T. laxa* Blue King were darker in color, the perianth segments did not spread so widely, and the leaves were slightly wider than those of *T. laxa*. In other characters, however, such as angle of attachment of the flowers, color of the stamens, and perhaps even chromosome number, the *T. laxa* Blue King plants were variable and intergraded with *T. laxa*. Such evidence indicates that *T. laxa* is a variable species, and that while chromosome counts may give a basis for separation into varieties, consistent morphological characters may not exist to make the separation possible.

Plants studied as *T. candida*, however, have both morphological and cytological characters which distinguish them from the *T. laxa* complex. The somatic number is 48, which is not an exact multiple of 7 as are the counts for *T. laxa*. The anthers of *T. candida* are only 2 mm. in length, while those of *T. laxa* are 3–4 mm. *T. candida* finished blooming before *T. laxa* had opened a single flower. The leaves of *T. candida* were yellowish green and 16 mm. wide; those of *T. laxa* were dark green and 5–10 mm. wide; those of *T. laxa* Blue King were 7–15 mm. wide. It is debatable whether "*candida*" is the correct specific name to use, since the flowers of the plant originally described as *T. candida* were white, but the need for separation from *T. laxa* is indicated.

The presence of an extra chromosome in the root-tip cells of one plant of *T. ixioides* var. *scabra* does not alter the fact that 10 is considered the typical somatic number. The fact that an extra fragment is present at meiosis lends interest to the situation. Figures 24 and 25 indicate that the increased number is not merely the result of the duplication of a single chromosome, nor could there have been a loss of a single chromosome from a complement of 12. The normal complement is made up of a pair of short chromosomes with a subterminal centromere, 4 chromosomes of medium length with submedian centromeres, and 4 long chromosomes—2 with a submedian centromere and 2 with an approximately median centromere. In the complement with an extra chromosome there are 4 long chromosomes and 4 of medium length, and in addition an extra long chromosome, a shorter than normal chromosome, and a chromosome of about the same length as the shortest in the normal complement. Such a situation could have arisen through duplication of a medium length or a small chromosome which subsequently became fragmented. One small fragment may have become attached to an end of one of the long chromosomes with a median centromere. Another fragment may have become attached to one of the shortest chromosomes. It is supposed that meiotic figures with an extra chromatin mass at metaphase I occurred in plants with a somatic number of 11, but there are no records to prove

the assumption. If the "fragment" did arise through duplication and fragmentation, it is strange that it was never observed associated at meiosis with one or both of the chromosomes of which it was originally a duplicate. Its small size is the most probable contributing cause. If the fragment possesses a centromere, it could be carried regularly through the process of sexual reproduction and be perpetuated. Also through vegetative propagation an irregularity of chromosome number could be retained in ever-increasing numbers of plants, regardless of whether or not they were capable of sexual reproduction. If the change had occurred far back in the history of the species, subsequent genic changes could account for the absence of multiple associations. This explanation of the presence of a chromosome fragment is largely hypothetical, but it is an application of NAVASHIN'S dislocation hypothesis (11) and is based on the known facts that trisomic plants may arise and that parts of chromosomes may be translocated to nonhomologous chromosomes.

Summary

1. The chromosome numbers of twenty-four species and varieties of *Brodiaea* have been investigated.

2. Somatic numbers of 10 (11), 12, 14, 16, 18, 28, 28 (30), 32, 36, 42, 48, and 50 chromosomes were observed. An incomplete series of corresponding gametic numbers includes 5 (6), 6, 8, 9, 14, 16, and 21.

3. Taxonomic and cytological findings seem to justify the separation of the material examined into three genera, *Brodiaea*, *Dichelostemma*, and *Triteleia*. It is suggested that the basic number for *Brodiaea* is 6, and for *Dichelostemma* the secondarily basic number 9, derived from 6; the variation of numbers in *Triteleia* is too great to postulate any one basic number.

4. The need for reconsideration of certain specific delimitations is suggested on the basis of cytological and taxonomic observations.

5. Variation of chromosome number is reported for two plants and an explanation for one of the variations offered on the basis of duplication of a chromosome and subsequent dislocation.

The writer acknowledges with sincere thanks the encouragement and guidance given by Dr. J. M. BEAL during this investigation. Completion of this work was made possible by the Fanny Bullock Workman Scholarship from Wellesley College.

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SEROLOGICAL STUDIES OF THE ERWINEAE. II. SOFT-ROT GROUP; WITH SOME BIOCHEMICAL CONSIDERATIONS

R. P. ELROD

Introduction

The taxonomy of the soft-rot group of organisms has had the attention of many workers in phytobacteriology. The group includes four species: *Erwinia carotovora* (Jones) Holland, *E. phytophthora* (Appel) Bergey *et al.*, *E. solanisapra* (Harrison) Holland, and *E. aroideae* (Townsend) Holland. Two other little known species are sometimes included, *E. flavida* (Fawcett) Magrou and *E. erivanensis* (Kalantarian) Bergey *et al.* Much evidence has accumulated to the effect that the group is but one variable species (**11, 21, 2, 8**), and numerous facts have also been offered for the separation of one or more species (**13, 12, 5, 10**). On the basis of previous work, however, it has become increasingly clear that the group at best is a heterogeneous one, and probably not susceptible to satisfactory classification.

Both *E. aroideae* and *E. solanisapra* have been declared to be variants of either *E. carotovora* or *E. phytophthora*. LEACH (**11**), in his list of synonyms for the blackleg pathogen, fails to list *E. aroideae*, but apparently he considers it the same as *E. carotovora*. BONDE (**2**) supports this idea, which was originally proposed by HARDING and MORSE (**8**). The latter workers considered it to be only an anaerogenic strain of *E. carotovora*. *E. solanisapra*, on the other hand, is considered by LEACH as synonymous with *E. carotovora*. MORSE (**17**) likewise concluded that *Bacillus atrosepticus*, *B. melanogenes*, and *B. solanisaprus* were but one species. He was supported in this contention by JENNISON (**9**).

From the extensive evidence, both pro and con, there seem to stand out two significant facts in favor of separating the soft-rot group into two distinct species: (1) results of inoculating into potato plants; and (2) maltose fermentation. According to LACEY (**10**) and DOWSON (**5**), if an organism can cause a soft-rot of potato accompanied by blackening of the stems, and if it ferments maltose, it is to be considered distinct from *Bacterium carotovorum*, which does not blacken potato stems or ferment maltose. This organism DOWSON calls *Bact. phytophthorum* (Appel) *comb. nov.* LEACH has contended that stem blackening cannot be accepted as a criterion for separation, since, according to his experiments with a representative group of soft-rot organisms, there occurred varying degrees of blackening, which offered no clear-cut differentiation. He concluded that it was impossible to separate one species from another on this basis. DOWSON (**4**), however, confuses

the issue still further by considering *Bact. aroideae* and *Bact. carotovorum* distinct, and lists the latter as "maltose−" and the former as "maltose+."[1]

It was deemed advisable to determine whether any correlation does exist between maltose+ and maltose− isolates and definite serological grouping. Before beginning the immunological studies, however, it was decided to ascertain whether or not there were any other fermentation data which might aid in either grouping all into a single species or separating into two species. At the same time, the antigenic structure of the organisms has been examined more thoroughly than heretofore. It was considered worthwhile also to ascertain the nature and specificity of extractable carbohydrate materials.

Cultures employed

The cultures employed[2] are as follows:

4.—*E. aroideae*, isolated from carrot, Brown University, R. P. Elrod, 1938.

5.—*E. aroideae*, isolated from carrot, Brown University, R. P. Elrod, 1938.

11.—*E. aroideae*, isolated from carrot, Brown University, R. P. Elrod, 1938.

P4.—*E. phytophthora*, isolated from potato, Ohio State University, R. P. Elrod, 1939.

ES.—*E. phytophthora*, isolated from *Delphinium ajacis* L., P. A. Ark, Berkeley, California, 1938.

NZ.—*E. melonis*, isolated from turnip, E. L. Cunningham, Auckland, New Zealand, 1939.

EA.—*E. aroideae*, isolated from pumpkin fruit, P. A. Ark, Berkeley, California, 1938.

CA1.—*E. carotovora*, University of Chicago, G. K. K. Link, 1939.

CA2.—*E. carotovora*, University of Chicago, G. K. K. Link, 1939.

EP.—*E. solanisapra*, A. T. C. C. no. 4668, 1939.

EMN.—*E. melonis*, A. T. C. C. no. 920, 1939.

496.—*E. phytophthora*, A. T. C. C. no. 496, 1940.

495.—*E. carotovora*, A. T. C. C. no. 495, 1940.

494.—*E. aroideae*, A. T. C. C. no. 494, 1940.

EC.—*E. carotovora*, Ohio State University stock culture, originally from University of Texas, 1938.

WV3.—*B. atrosepticus*, originally from United States Department of Agriculture, obtained through A. R. Stanley, University of West Virginia, 1940.

[1] Throughout this paper, maltose− refers to the failure to ferment maltose, while maltose+ indicates the formation of acid, or acid and gas.

[2] The writer is indebted to Drs. P. A. Ark, G. K. K. Link, E. L. Cunningham, and A. R. Stanley for some of the cultures employed.

WV4.—*B. dissolvens*, originally from United States Department of Agriculture, obtained from A. R. Stanley, University of West Virginia, 1940.

WV6.—*B. dissolvens*, isolated from stalk rot of sweet corn, A. R. Stanley, University of West Virginia, 1940.

All these cultures proved pathogenic on either carrot or potato slices, some to a far greater degree than others. Each organism in the list bears the name under which it was received. Several of these are now considered synonymous with one of the four accepted species. *E. melonis* (Giddings) Holland is now declared to be a synonym of *E. aroideae* (CHESTER, in BERGEY *et al.*, 1). *Bacillus dissolvens* is said to be identical with *E. carotovora* (20, 1), while *B. atrosepticus* is considered synonymous with *E. phytophthora* (11, 1).

I. Fermentation experiments

METHODS

Fermentative studies of the soft-rot group have seldom been undertaken with any completeness. Except for the more common carbohydrates, our knowledge of the fermentative abilities of these organisms has been meager. STAPP (21) made a detailed study of the fermenting capacities of many of the soft-rot organisms, but this was done before the group, as now understood, had been formulated. With this in mind, it was deemed advisable to employ as many fermentable substances as were on hand. These totaled twenty-six carbohydrates, higher alcohols, and glucosides.

Durham fermentation tubes were employed. The medium was nutrient broth to which was added enough of the fermentable substance to make a solution of 1 per cent. Brom-cresol-purple was used as an indicator. The sugars were sterilized by filtration through either a Seitz or Berkefeld filter and added aseptically to the sterile broth. Tubes were inoculated from fresh agar cultures and observed over a period of 5 weeks. The experiments were performed in triplicate.

RESULTS

Table 1 shows how active all the isolates were in utilizing the majority of the carbohydrates. Lactose, sucrose, dextrose, arabinose, galactose, inositol, levulose, mannitol, salicin, mannose, and glycerol were fermented in each instance. Erythritol, melezitose, inulin, dextrin, and dulcitol were fermented in rare cases. Sorbose and adonitol were attacked by none of the eighteen isolates. Eight cultures fermented maltose, while six broke down sorbitol.

On the criterion of gas production, the eighteen cultures formed roughly three groups: (*a*) those that formed only acid in any of the media (EC, 5, 11, 4, EA, EMN, WV3, and 494); (*b*) those that produced a small, immeasurable quantity

TABLE 1*

BIOCHEMICAL REACTIONS

Organism tested	Dextrose	Sorbose	Galactose	Levulose	Mannose	Arabinose	Xylose	Melibiose	Maltose	Lactose	Sucrose	Cellobiose	Rhamnose	Trehalose	Melezitose	Raffinose	Adonitol	Sorbitol	Inositol	Dulcitol	Mannitol	Erythritol	Glycerol	Salicin	Amygdalin	Inulin
EC	A	−	A	A	A	A	A	A	−	A	A	A	A	A	−	A	−	−	A	−	A	−	A	A	A	−
5	A	−	A	A	A	A	A	A	−	A	A	A	A	A	−	A	−	−	A	−	A	−	A	A	A	−
11	A	−	A	A	A	A	A	A	−	A	A	A	A	A	−	A	−	−	A	−	A	−	A	A	A	−
4	A	−	A	A	A	A	A	A	−	A	A	A	A	A	−	A	−	−	A	−	A	−	A	A	A	−
EA	A	−	A	A	A	A	A	A	−	A	A	A	A	A	−	A	−	−	A	−	A	−	A	A	A	−
EMN	A	−	A	A	A	A	A	A	−	A	A	A	A	A	−	A	−	−	A	−	A	−	A	A	A	−
WV3	A	−	A	A	A	A	A	A	−	A	A	A	A	A	−	A	−	−	A	−	A	−	A	A	A	−
494	A°	−	A	A	A	A	A°	A°	−	A°	A	A	A°	A	−	A°	−	−	A°	−	A	−	A	A	A	−
NZ	A°	−	A°	A°	A	A	A°	A°	−	A°	A	A	A°	A°	−	A°	−	−	A°	−	A	−	A	A	A	−
495	A°	−	A°	A°	A	A	A°	A°	−	A°	A	A	A°	A°	−	A°	−	−	A°	−	A	−	A	A	A	−
ES	A°	−	A°	A°	A°	A	A	A	−	A°	A	A°	A	A	−	A	−	−	A°	−	A	−	A	A	A	−
496	A	−	A	A	A°	A	A	A	−	A°	A	A°	A	A	−	A	−	−	A°	−	A	−	A	A	A	−
P4	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	−	+	+	−	+	−	+	+	A	+
EP	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	−	+	+	−	+	−	+	+	A	+
CA1	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	A°	−	+	−	+	+	A	+
CA2	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	A°	−	+	−	+	+	A	+
WV4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	−	A	+	A	−
WV6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	−	+	−	A	+	A	−

* +, Acid and measureable gas; A°, acid and bubble of gas; A, acid only; −, no acid or gas.

of gas in some media, often only a small bubble that dissolved into the liquid (NZ, ES, 496, and 495); and (*c*) those that produced at least 10 per cent gas in the vial (P4, EP, CA1, CA2, WV4, and WV6). All the last group fermented maltose, while none of the acid-forming group attacked this disaccharide. Two of the intermediate group (ES and 496) also fermented maltose, with the formation of acid only, while the two other members (495 and NZ) failed to do so.

The culture NZ was a fairly recent isolate (2 months) when tested and probably never had the ability to form abundant gas. Culture ES has had a peculiar history. When first received it produced abundant gas in lactose, dextrose, and sucrose, but this property was lost while the culture was continued in stock. Experiments, however, proved that this loss of gas production did not parallel an antigenic change. Both 495 and 496 had been carried in stock for years and might possibly have lost the ability to form gas abundantly. It is probably also worthy of note that 496 was originally identified as *B. phytophthorus* by Appel, from whom it was received in 1906 by the Bureau of Plant Industry, United States Department of Agriculture. Culture 495, on the other hand, was received from L. R. Jones in 1920 by the American Type Culture Collection as a culture of *E. carotovora*. The history of the latter two organisms is interesting in view of the fact that 496 is maltose+ and 495 is maltose−.

The writer supports the contention that *E. aroideae* is synonymous with *E. carotovora*. In the present small series of fermentation tests are found every intergradation of gas production, and also evidence that a strong aerogenic organism can become almost anaerogenic. It seems improbable that a definite division can be made on the basis of gas production. STANLEY (**19**) recorded sixty-seven changes in the fermentative capacity of forty-three organisms in the breakdown of dextrose, lactose, and sucrose. These changes included the inability to ferment one or more of those sugars as well as changes from an aerogenic to an anaerogenic state, or vice versa.

In the maltose-fermentation experiment this substance was broken down by all the gas-producers and some of the anaerogenic strains. Two of the strains, NZ and 495, which fermented many carbohydrates with the formation of a small quantity of gas, did not ferment maltose. In subsequent experiments NZ was proved to be identical antigenically with a strain of *E. aroideae* (EA), which is a strictly anaerogenic organism. The case of 495 has already been mentioned. If it is in reality representative of the *E. carotovora* group, it would be expected not to ferment maltose (5). Most of the organisms at my disposal, however, considered to be *E. carotovora* or a synonym of it, fermented maltose.

Of all the substances used, only one other (sorbitol) seems to offer anything for satisfactory use in the way of a natural separation. As can be seen in table 1, all the abundant gas-producers fermented sorbitol, while none of the anaerogenic or

meager gas-formers broke it down. Sorbitol fermentation experiments have been previously reported in this group by STAPP (**21**). He obtained negative results with all his strains, which included *Bacillus phytophthorus*, *B. atrosepticus*, *B. carotovorus*, and *B. solanisaprus*. Two organisms (ES and 496), which fermented maltose with the production of acid only, failed to attack sorbitol. But for these reactions, there was absolute agreement between the maltose and sorbitol fermentation. It is entirely possible that sorbitol fermentation is of considerable significance. This can be determined only by a more exhaustive study.

II. Serological results

Immune sera were prepared against thirteen of the eighteen cultures: four anaerogenic strains—4, EA, EMN, and 11 (all maltose−); three which produced small quantities of gas—NZ (maltose−), 496, and ES (maltose+); and all six of the gas-producing organisms—P4, EP, CA1, CA2, WV4, and WV6 (all maltose+).

The results of the cross-agglutination tests are found in table 2. It is evident that there was a fair degree of cross-agglutination throughout the whole group, since there was a total of forty reactions out of a possible two hundred and twenty-one, or 18.1 per cent. This is exclusive of the homologous reactions. Only those reactions which attained at least a titer of 1:40 were considered. The average homologous titer of the thirteen sera employed was 22,110, while the forty heterologous reactions had an average titer of 2950. The titers of the cross-reactions ranged between 80 and 20,480. Normal sera drawn in every case failed to agglutinate any of the organisms in a dilution of 1:20 or greater.

If, however, the maltose+ and the maltose− organisms are considered separately, a far higher degree of correlation is obtained. The five sera prepared against the non-maltose fermenting organisms agglutinated 33.3 per cent (fifteen of a possible forty-five) of the maltose− group. The average homologous titer of these sera was 13,312, while the fifteen heterologous titers averaged 2251. On the other hand, the eight sera prepared against maltose+ organisms produced 28.6 per cent (sixteen of a possible fifty-six) cross-reactions with the maltose fermentors. The average homologous titer in this case was 27,885 and the heterologous average was 4165.

Although the percentage of cross-reaction was higher in the maltose− group, three organisms, WV3, 494, and 495, failed to react with any of the sera. The percentage of cross-reactions would doubtless have been lower had sera been prepared against one or more of these organisms. The maltose+ organisms all showed some degree of cross-agglutination within that group.

Probably just as significant as the fairly high percentage of cross-reaction within the fermentative groups, if not more so, was the very small amount of cross-agglutination found among them. There were but nine reactions out of a possible

one hundred and twenty, or only 7.5 per cent. Hence the bulk of the 18.1 per cent cross-reaction occurred within the maltose+ and maltose− groups. Four of the nine crosses were due to the borderline organism, ES, which produces only acid in maltose and serologically is extremely close to two members of the maltose− group. Subsequent adsorption experiments have shown EA and NZ to be identical and ES to contain an antigenic component common to these two.

TABLE 2*

RESULTS OF CROSS-AGGLUTINATION EXPERIMENTS

ORGANISM AGGLUTINATED	IMMUNE SERA PREPARED AGAINST												
	MALTOSE−					MALTOSE+							
	4	EA	EMN	11	NZ	ES	496	P4	EP	CA1	CA2	WV4	WV6
4	20480	80	320	10240	−	−	−	160	−	−	−	−	−
EC	−†	−	−	−	80	−	−	−	−	−	−	−	−
EA	−	10240	−	80	10240	5120	−	−	−	−	−	−	−
5	640	−	160	640	−	−	−	−	−	−	−	−	−
EMN	80	−	5120	160	−	−	160	−	−	−	−	−	−
11	320	160	320	20480	−	−	−	160	−	−	−	−	−
WV3	−	−	−	−	−	−	−	−	−	−	−	−	−
494	−	−	−	−	−	−	−	−	−	−	−	−	−
NZ	−	10240	−	−	10240	5120	−	−	−	−	−	−	−
495	−	−	−	−	−	−	−	−	−	−	−	−	−
ES	−	640	−	−	5120	40960	−	−	−	−	−	−	−
496	−	−	−	−	−	−	5120	−	−	−	−	5120	−
P4	640	−	−	320	−	640	−	20480	160	2560	80	−	−
EP	−	−	−	−	−	320	−	−	40960	−	−	5120	20480
CA1	−	−	−	−	−	160	−	5120	−	20480	1280	−	−
WV4	−	−	−	−	−	−	2560	−	5120	−	−	10240	−
WV6	−	−	−	−	−	−	−	−	10240	−	−	−	81920

* Normal serum and saline controls negative. All titers referred to as their reciprocals.
† −, less than 1:40.

Adsorption experiments were conducted in the majority of the cases in which cross-agglutination occurred. The numerous experiments were not informative. It could be definitely shown that for the most part the various agglutinative groups did possess common factors, which manifested themselves on adsorption by causing a reduction of the homologous titer. By means of these experiments it was possible to identify twenty-two different flagellar components in the eighteen organisms. Their pattern, however, offered no hope of forming definite serological groups.

During the course of agglutination and agglutinin-adsorption investigations, a situation arose that was felt to need further study. The organism EP has been shown to be virtually analogous to CA1, CA2, and P4 from a biochemical stand-

point. To be sure there were certain differences, but these apparently were not significant. Serologically, EP showed little relationship to these three organisms. Only in the case of P4, which agglutinated to a titer of 160 in EP antiserum, was there evidence of cross-reaction. The reason for this unilateral expression was difficult to determine.

With this in mind, adsorption tests were carried out with EP on P4 antiserum. As can be seen in table 3, all the cross-reacting agglutinins were removed and the homologous titer was reduced from 20,480 to 1280. Repeated adsorptions failed to reduce this latter figure. The reciprocal adsorption P4, with EP antiserum, was less spectacular because of the lack (except WV6) of heterologous reactors. The homologous titer, however, was reduced from 40,960 to 20,480. On such a basis it

TABLE 3

ADSORPTION EXPERIMENTS WITH ANTI-P4 SERUM

ORGANISM AGGLUTINATED	BEFORE ADSORPTION	ADSORBED WITH					
		11	4	CA2	CA1	EP	P4
P4	20480	5120	2560	1280	1280	1280	—*
CA1	5120	5120	5120	—	—	—	—
CA2	2560	1280	1280	—	—	—	—
11	160	—	—	160	80	—	—
4	160	—	—	80	80	—	—

* —, Less than 1:40.

would be easy to conclude that EP does have factors in common with P4, which do not manifest themselves by direct agglutination. EP likewise reduced to a considerable extent the homologous and heterologous titers in CA1 and CA2 sera.

NATURE OF CROSS-REACTION

Virtually all the cross-agglutination that occurred with the soft-rot organisms took place in a floccular manner. This type of reaction has been shown to be due, in other groups of bacteria, to flagellar components and their corresponding agglutinins. That this is also true in the soft-rot group can be shown by the following experiment.

It was pointed out by WHITE (**22**) that the flagellar components of *Salmonella* organisms were destroyed, with no impairment to the somatic fractions, by growing the organisms on phenol agar (1:1000) and then heating the growth in a water-bath at 100° C. for 30 minutes. Such suspensions are homogeneous and agglutinate only in a coarse, granular fashion, indicative of reaction between somatic components and corresponding agglutinins. The commonly used alcohol precipitation method did not give satisfactory results with the *Erwinia*.

Inasmuch as the antisera contained both flagellar and somatic agglutinins, in order to obtain a serum which affected only the flagellar components, all that was necessary was to absorb any one serum with the homologous phenol-heated antigen. After several such adsorptions, all the somatic antibodies were removed. There was, however, no loss of titer of the cross-reactions, when such an adsorbed serum was used in agglutination tests. This indicated conclusively that the cross-reactions in the group are due to common flagellar components (table 4).

TABLE 4

RESULTS OF FLAGELLAR CROSS-AGGLUTINATION EXPERIMENTS

ORGANISM AGGLUTINATED	IMMUNE SERA PREPARED AGAINST AND ADSORBED WITH HOMOLOGOUS PHENOL-HEATED ORGANISMS												
	4	EA	EMN	11	NZ	ES	496	P_4	EP	CA_1	CA_2	WV_4	WV6
4	5120	80	320	10240	—	—	—	160	—	—	—	—	—
EC	—*	—	—	—	—	—	—	—	—	—	—	—	—
EA	—	—	—	—	80	—	—	—	—	—	—	—	—
5	640	—	160	640	—	—	—	—	—	—	—	—	—
EMN	80	—	2560	160	—	—	320	—	—	—	—	—	—
11	320	160	320	20480	—	—	—	160	—	—	—	—	—
WV_3	—	—	—	—	—	—	—	—	—	—	—	—	—
494	—	—	—	—	—	—	—	—	—	—	—	—	—
NZ	—	5120	—	—	10240	5120	—	—	—	—	—	—	—
495	—	—	—	—	—	—	—	—	—	—	—	—	—
ES	—	640	—	—	5120	10240	—	—	—	—	—	—	—
496	—	—	—	—	—	—	5120	—	—	—	—	5120	—
P_4	640	—	—	320	—	640	—	5120	160	2560	80	—	—
EP	—	—	—	—	—	320	—	—	20480	—	—	5120	20480
CA_1	—	—	—	—	—	160	—	2560	—	1280	1280	—	—
CA_2	—	—	—	—	—	—	—	1280	—	5120	1280	—	—
WV_4	—	—	—	—	—	—	2560	—	5120	—	—	10240	—
WV6	—	—	—	—	—	—	—	—	5120	—	—	—	81920

* —, less than 1:40.

Experimentation with sera prepared against phenol-heated antigens revealed the opposite of the results found with the flagellar components. With either living or phenol-heated antigens, there was little evidence of cross-agglutination (table 5). Only in cases where cross-adsorption tests revealed that the organisms were identical, or nearly so, were there cross-reactions. The agglutination tests for somatic components were incubated 18 hours at the normal temperature. The titers prepared against phenol-heated antigens were also exceedingly low. Such results are in accord with those of WHITE with *Salmonella* somatic sera.

Adsorption experiments were also conducted with respect to the somatic components. The bulk of the adsorptions were carried out with fresh packed cells, although some were done with phenol-heated organisms. Inasmuch as the flagellar components were of no consequence, the use of untreated packed cells was more

convenient. The data from such experiments are meager because of but a minimum of somatic cross-agglutination. In the case of NZ and EA, however, it was demonstrated that the components in each were the same. This was also deducible from straight adsorption experiments. In the CA1, CA2, and P4 series it was shown that CA2 had a common somatic component with CA1, but that the latter possessed one in excess. This was the only determinable serological difference between CA1 and CA2, inasmuch as their flagellar components were the same.

TABLE 5

RESULTS OF SOMATIC CROSS-AGGLUTINATION EXPERIMENTS

ORGANISM AGGLUTINATED	IMMUNE SERA PREPARED AGAINST PHENOL-HEATED ORGANISMS								
	4	EA	EMN	NZ	ES	P_4	EP	CA_1	CA_2
4	5120	—	80	—	—	—	—	—	—
EC	—*	—	—	—	—	—	—	—	—
EA	—	80	—	320	320†	—	—	—	—
5	640	—	—	—	—	—	—	—	—
EMN	320(?)	—	640	—	—	—	—	—	—
11	1280	—	40	—	—	—	—	—	—
WV3	—	—	—	—	—	—	—	—	—
494	—	—	—	—	—	—	—	—	—
NZ	—	80	—	320	160†	—	—	—	—
495	—	—	—	—	—	—	—	—	—
ES	—	—	—	80†	640	—	—	—	—
496	—	—	—	—	—	—	—	—	—
P_4	—	—	—	—	—	320	—	80	—
EP	—	—	—	—	—	—	5120	—	—
CA_1	—	—	—	—	—	—	—	320	160
CA_2	—	—	—	—	—	—	—	160	320
WV4	—	—	—	—	—	—	—	—	—
WV6	—	—	—	—	—	—	—	—	—

* —, less than 1:40. † Floccular; all others granular.

It is obvious that there were too few opportunities for experimentation with somatic components. The 4, 11, 5, and EMN group likewise showed variance in somatic factors. That such factors were not absolutely specific for each organism is to be expected. It is hardly conceivable that each type would possess an individual somatic component. It is fairly certain, however, that the somatic components are far more type specific than are those of the flagella.

Carbohydrate materials were extracted from all the organisms of the soft-rot group. In every case the material gave a strong Molisch test for the furan ring (indicative of some type of polysaccharide) and yielded a negative or weakly positive Biuret reaction. Those weakly positive could be made negative through further purification by a second acetone precipitation. The carbohydrate material was also found able to resist tryptic digestion with no impairment of its precipitative ability.

The material was highly reactive in precipitation experiments, giving strong ring tests even when diluted (in some cases) to 1:100,000. The titers differed in some respects according to the individual sera. Even with low titering agglutinating sera, a strong precipitin test was often observed. With one serum (EAO) prepared after the usual course of injections, which exhibited an agglutination titer of 1:80, a good precipitin result was obtained.

The result of the precipitin tests (table 6) paralleled in every respect the somatic agglutination. It therefore became desirable to ascertain whether it was the

TABLE 6*

PRECIPITATION CROSS-REACTIONS WITH EXTRACTED CARBOHYDRATE MATERIALS

Organism precipitated	Immune sera prepared against											
	4	EA	EMN	NZ	ES	P_4	EP	CA_1	CA_2	496	WV6	WV_4
4	+	−	±	−	−	−	−	−	−	−	−	−
EC	−	−	−	−	−	−	−	−	−	−	−	−
EA	−	+	−	+	−	−	−	−	−	−	−	−
5	+	−	−	−	−	−	−	−	−	−	−	−
EMN	±	−	+	−	−	−	−	−	−	−	−	−
11	+	−	−	−	−	−	−	−	−	−	−	−
WV_3	−	−	−	−	−	−	−	−	−	−	−	−
494	−	−	−	−	−	−	−	−	−	−	−	−
NZ	−	+	−	+	−	−	−	−	−	−	−	−
495	−	−	−	−	−	−	−	−	−	−	−	−
ES	−	−	−	−	+	−	−	−	−	−	−	−
496	−	−	−	−	−	−	−	−	−	+	−	−
P_4	−	−	−	−	−	+	−	−	−	−	−	−
EP	−	−	−	−	−	−	+	−	−	−	−	−
CA_1	−	−	−	−	−	−	−	+	±	−	−	−
CA_2	−	−	−	−	−	−	−	±	+	−	−	−
WV_4	−	−	−	−	−	−	−	−	−	−	−	+
WV6	−	−	−	−	−	−	−	−	−	−	+	−

* +, Strong reaction; ±, weak reaction; −, no reaction.

same reactive material in each case. Experiments showed this to be so. For example, extraction of material from phenol-heated cells which had been carefully washed gave results identical with those of whole cell extractions. Likewise, carbohydrate antigens precipitated strongly with sera prepared against phenol-heated antigens.

Adsorption experiments also showed the carbohydrate material to be bound up in the somatic portion of the cell. The employment of sera which had been prepared for testing flagellar agglutinations resulted in negative precipitin tests. This in spite of the fact that there still remained strong flagellar agglutinating factors.

Discussion

Although it has been apparent for a number of years that the soft-rot group of organisms is exceedingly heterogenetic from a serological standpoint (3, 10, 14,

15, 16, 19, 21), it should not be considered improbable that definite serological groups might be encountered. It is apparent from this report that such a division as maltose fermentation might well divide the soft-rot group into two quite compact serological groups. It is realized, however, that the number of cultures used in these experiments was small. Nevertheless, the fact remains that there is a high percentage of cross-agglutination within the groups. This percentage is far higher than might possibly occur by chance, as can be proved by applying statistical methods. It is the contention of the writer that the comparatively high percentage of cross-reaction found within the maltose+ and maltose− groups, together with the resulting high titers, is indicative of considerable serological homogeneity. Experimentation carried on at the present time has revealed many common antigenic components. This is especially true within the two fermentative groups, although there is evidence of common antigenic factors among maltose+ and maltose− groups.

It is possible also that the high degree of serological heterogeneity is due to the presence of numerous inagglutinable strains. If these organisms were used for adsorption, however, their relationship would become evident and a far higher degree of serological homogeneity would result. Hence it is apparent that EP belongs in the CA_1, CA_2, P_4 group, although it fails to agglutinate in any of the corresponding sera. A similar situation has been recorded in the *Salmonella* by WHITE (23).

The present experiments have shown that the flagellar components of the soft-rot organisms are group specific in nature, whereas the somatic elements are apparently type specific. It has been pointed out by others that the flagellar components of the genus *Salmonella* are type specific in nature and the somatic fractions group specific. In the genus *Proteus*, however, this situation is reversed: the common antigenic fractions are flagellar and the somatic elements are type specific. It is evident then that my group is analogous to the *Proteus* in its antigenic makeup. This is further emphasized on comparing the action of extracted carbohydrate materials. FURTH and LANDSTEINER (7) have demonstrated that, in the *Salmonella*, carbohydrate materials are derived from the somatic portion of the cell and immunologically act in the group-specific manner as do the somatic antigens. ELROD (6) has found this to be true of *E. amylovora*.

PRZESMYCKI (18) has shown the situation to be different in the genus *Proteus*. Here extracted carbohydrate materials are specific, even as the somatic elements. It may be that the serological heterogeneity found in the *Erwinia* and the *Proteus* groups is in some way linked with the flagellar components being group specific.

Summary and conclusions

1. Eighteen cultures of *Erwinia carotovora* (soft-rot group) were tested for fermentative ability in twenty-six substances. Division on the basis of maltose fer-

mentation found ten fermenting the sugar with the formation of either acid or acid and gas, while eight failed to ferment the carbohydrate. The maltose− organisms were agglutinated in 33.3 per cent of the cases with maltose− antisera. There was 28.6 per cent of cross-reaction with the maltose+ cultures and maltose+ antisera. Between the two fermentative groups there was but 7.5 per cent of cross-reaction. The total number of cross-agglutination experiments produced with thirteen antisera was 18.1 per cent, the majority of which occurred within the two fermentative groups.

2. A division on the basis of sorbitol fermentation may be significant and is considered worthy of further investigation.

3. The correlation between maltose fermentation and serological groups seems to bear out the contention of others that there are two soft-rot pathogens. These two groups, however, show within them considerable variation in fermentative powers and antigenic structure.

4. The common antigenic components of the group are bound up in the flagella. Adsorption experiments showed a large number of flagellar components.

5. The somatic fractions were shown to be type specific. There was, however, some evidence of common somatic factors.

6. Carbohydrate materials extracted from the organisms proved to be specific, and paralleled the somatic results. In all probability the somatic fractions owe their specificity to these carbohydrate materials.

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EFFECT OF BORON ON GROWTH AND DEVELOPMENT OF THE RADISH

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 531

JOHN SKOK

(WITH FIVE FIGURES)

Introduction

AGULHON (1) found that different species of plants reacted differently toward boron, and that for each there was an optimum concentration which favored growth and yield of the plant, although he did not describe the deficiency symptoms in detail. Later HASELHOFF (14) reported that boron increased growth in beans; BRENCHLEY (3) found peas to grow better with boric acid; and MAZÉ (20) found boron was required by maize and added it to his list of essential elements. WARINGTON (29) and SOMMER and LIPMAN (28) showed definitely that boron was essential for the normal growth of the broad bean and a number of other plants. Several excellent summaries are available (4, 12, 15, 22, 33) of more recent work on this subject.

The exact role of boron is still not known. It has been possible merely to speculate as to its function by noting the development of plants when it is absent. Much has been learned from work done during the past 20 years concerning gross deficiency symptoms and histological and metabolic effects. PURVIS (24) has reviewed the effects of boron and the factors affecting its deficiency in the soil. In general, boron-deficient plants show disintegration of the meristematic and conducting tissues and the eventual death of first the growing tip and finally the entire plant, retarded root development, and brittleness of the leaf blades and petioles. EATON (8) found that the effects of boron deficiency are first noticeable in the youngest and of boron excess in the oldest portions of the plant. Some investigators (7, 21) think boron functions as a hormone or enzyme or is essential to the formation of substances which are activators or regulators of metabolic processes, while others (6, 18, 29) question the hormonal or enzymatic role. There are some indications that boron may have an effect on calcium absorption (6, 32) and utilization (19, 27); and microchemical studies by SHIVE (26) suggest that it is intimately involved in protein metabolism. Both ammonium nitrogen and sugar accumulate in minus boron plants. This strongly suggests that boron deficiency interferes with the aminization of the carbohydrate derivatives and in this way interferes with metabolic activity in meristematic regions.

In the present experiments a study was made of the effect of boron on the

growth and development of the radish. AGULHON (**1**) included the radish in his studies and found a definite increase in growth with boron, but he did not describe the deficiency symptoms. SHIVE (**26**) found definite deficiency symptoms in the Long Icicle variety 6 days after germination. The first foliage leaves became discolored and distorted, and finally the growing tips died. He did not describe symptoms of the hypocotyl or root. PURVIS and HANNA (**25**) found that among a number of other plants the radish showed boron deficiency symptoms in various fields in Virginia. They state that the symptoms consisted of root malformations and an internal darkening very similar to that found in turnips.

Material and methods

Seeds of *Raphanus sativus* L., variety Scarlet Globe, were planted in flats of clean quartz sand, and after 10–12 days plants of uniform size were transplanted to glazed earthenware pots filled with the same material. All plants received a minus boron nutrient until after transplanting and then received applications of the appropriate nutrient solutions every second day. The solutions were made up with distilled water and Merck's reagent quality chemicals of the following concentrations: 0.006*M* $Ca(No_3)_2$; 0.0045*M* KH_2PO_4; 0.0045*M* $MgSO_4$; 0.5 p.p.m. Fe added as ferric citrate; 0.5 p.p.m. Mn added as $MnCl_2$; 0.5 p.p.m. Zn added as $ZnCl_2$; and 0.125 p.p.m. Cu added as $CuCl_2$. The plus boron solution received in addition 0.5 p.p.m. B added as H_3BO_3. The plants were harvested 39–42 days after the seeds were planted. Fresh and dry weights of the top and root portions and volumes of the root portions were taken. The volumes were determined by the water displacement method. The term root as used here includes the enlarged underground portion which consists of the hypocotyl as well as the root. Percentage dry weights and top-root ratios were also determined.

Experimentation

These experiments were of three types: (*A*) a study of the gross boron deficiency symptoms of the radish, (*B*) a study of the effect of photoperiod on the manifestation of the deficiency symptoms, and (*C*) a histological study.

A. Gross boron-deficiency symptoms

Three series of plants were grown under different treatments. The plants of series 1 received boron throughout the period of the experiment. The seeds were planted April 6, 1939, and the plants were harvested 41 days later. The plants of series 2 received boron for 18 days after planting, at which time the plants were 3–4 cm. tall and had four leaves. From then on they received a minus boron nutrient. They were planted and harvested at the same time as the plants in series 1. The plants of series 3 were grown without boron throughout the experi-

ment. They were planted May 12, 1939, and harvested 39 days later (table 1, fig. 1).

The plus boron plants of series 1 had large green leaves 15–20 cm. long and 5–6 cm. broad. The thickened portions were large, bright red, solid, and ranged from 2 to 4 cm. in diameter.

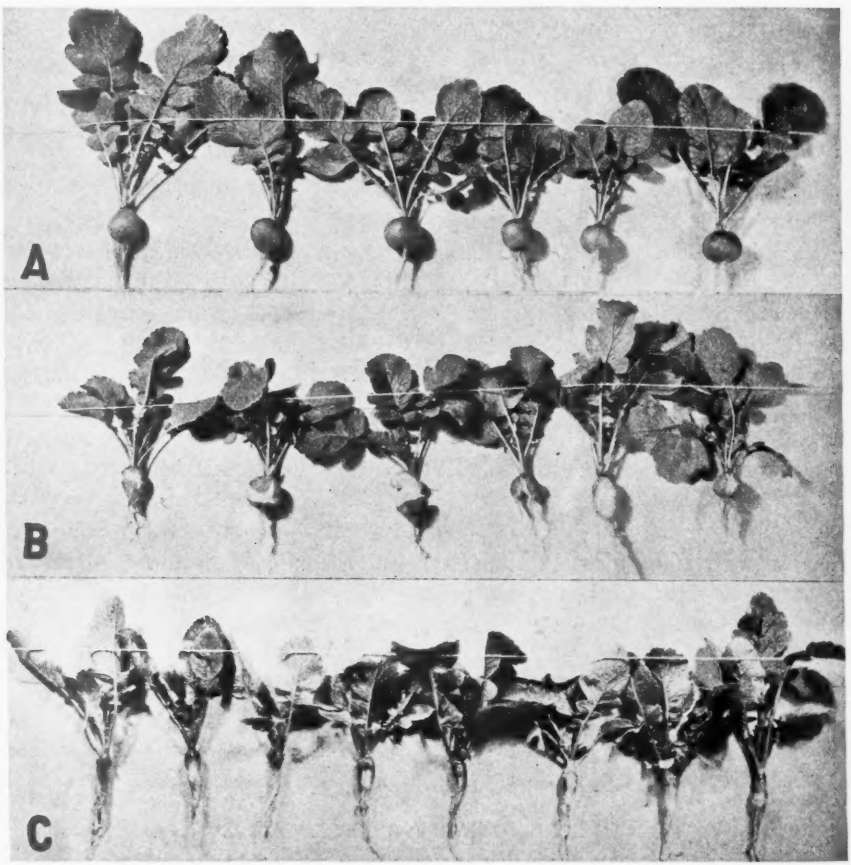

FIG. 1.—*A*, series 1, plus boron plants; *B*, series 2, plus boron for first 18 days only; *C*, series 3, minus boron plants.

The top portions of the plants in series 2, which received boron for the first 18 days, were well developed and similar to those in the first series, although the leaves were slightly lighter and some were distinctly mottled. The plants were also slightly smaller and the petioles were rather brittle as compared with those of the plus boron plants. The percentage dry weight was nearly the same. The

enlarged portions of this series were all light in color, and all of them were much cracked. The splitting was usually longitudinal but in some cases was also transverse. The cracks in some cases were shallow and in others were very deep; a few radishes were split almost in half. The size of the thickened portion was slightly smaller than that of the plus boron series, as is shown in table 1.

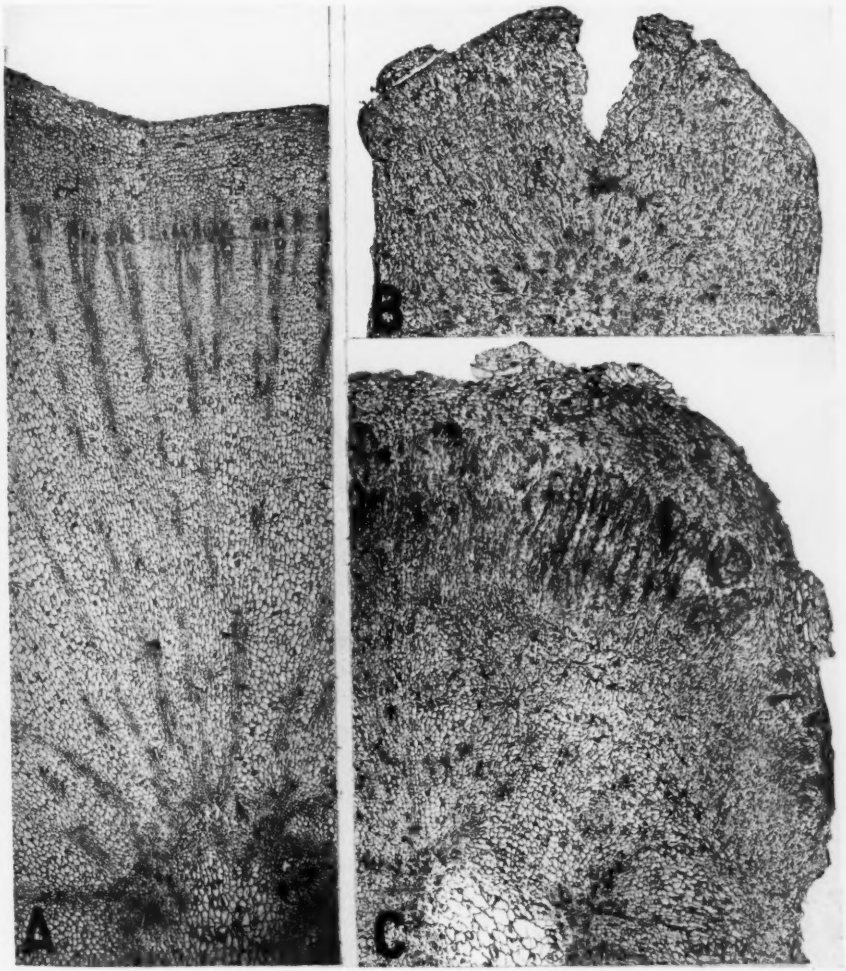

FIG. 2.—Section through hypocotyl region: *A*, plus boron; *B*, *C*, minus boron. *B* and *C* show unaffected vascular tissue at central portion of axis and absence of lignified vessels as well as of disintegrated phloem in area between central vascular tissue and cambium region. Split section (*B*) shows indistinctly developed vascular bundles, and unsplit section (*C*) shows dome arrangement of bundles at cambium region.

The plants of series 3, which were continuously on a minus boron nutrient, were much smaller than either of the other groups. The leaves were 8–15 cm. long, very pale, some extremely chlorotic, the petioles brittle and easily broken when handled. The chlorosis was confined to the areas between the veins, the veins themselves being usually green. Some of the older leaves were curled downward, but generally they were not markedly deformed. The young leaves in the center were very small, curled, thick, and usually brittle. The total growth of the tops as measured by dry weight was not much less than that in the plus boron series, but the total root growth was only about half as great. The percentage dry weight was also greater in this group than in either of the other two. The hypocotyl and

TABLE 1

DATA OF EXPERIMENTS OF PART A. VOLUMES AND WEIGHTS REPRESENT AVERAGES PER PLANT ON 40-PLANT BASIS

CONDITION	VOLUME OF ROOT (ROOT AND HYPOCOTYL) (CC.)	WET WEIGHT		DRY WEIGHT		PERCENTAGE DRY WEIGHT			WET TOP-ROOT RATIO	DRY TOP-ROOT RATIO
		TOP (GM.)	ROOT (GM.)	TOP (GM.)	ROOT (GM.)	ENTIRE PLANT	TOPS	ROOTS		
Series 1 (plus boron)...	10.19	6.27	10.19	0.64	0.63	7.75	10.22	6.22	0.62	1.01
Series 2 (plus boron for first 18 days)	8.92	5.36	8.71	0.61	0.54	8.17	11.38	6.20	0.61	1.13
Series 3 (minus boron).	4.19	5.32	4.09	0.57	0.30	9.25	10.71	7.33	1.30	1.90

root portions of the plants in this series were much elongated, narrow, and all were nearly white in color, and split. The splitting here also was usually parallel with the axis but in some cases at right angles to it. The volumes of the thickened portions of the plants in this series were less than half of those of the plus boron series and of an entirely different shape. The volumes of the thickened portions in series 2 were only slightly smaller that those of the plus boron series, and aside from the splitting, were of about the same shape.

A common boron-deficiency symptom is death of the growing tip and cessation of further top growth. The plants in these experiments showed only moderate symptoms in the tops but marked symptoms in the underground portions. SHIVE (**26**) reported that lack of boron resulted in complete destruction of the growing tip in radishes grown in sand which was treated with acid. The sand used in these experiments was not so treated and may have contained minute traces of boron. The boron-deficient radishes reported by PURVIS and HANNA (**25**) showed symp-

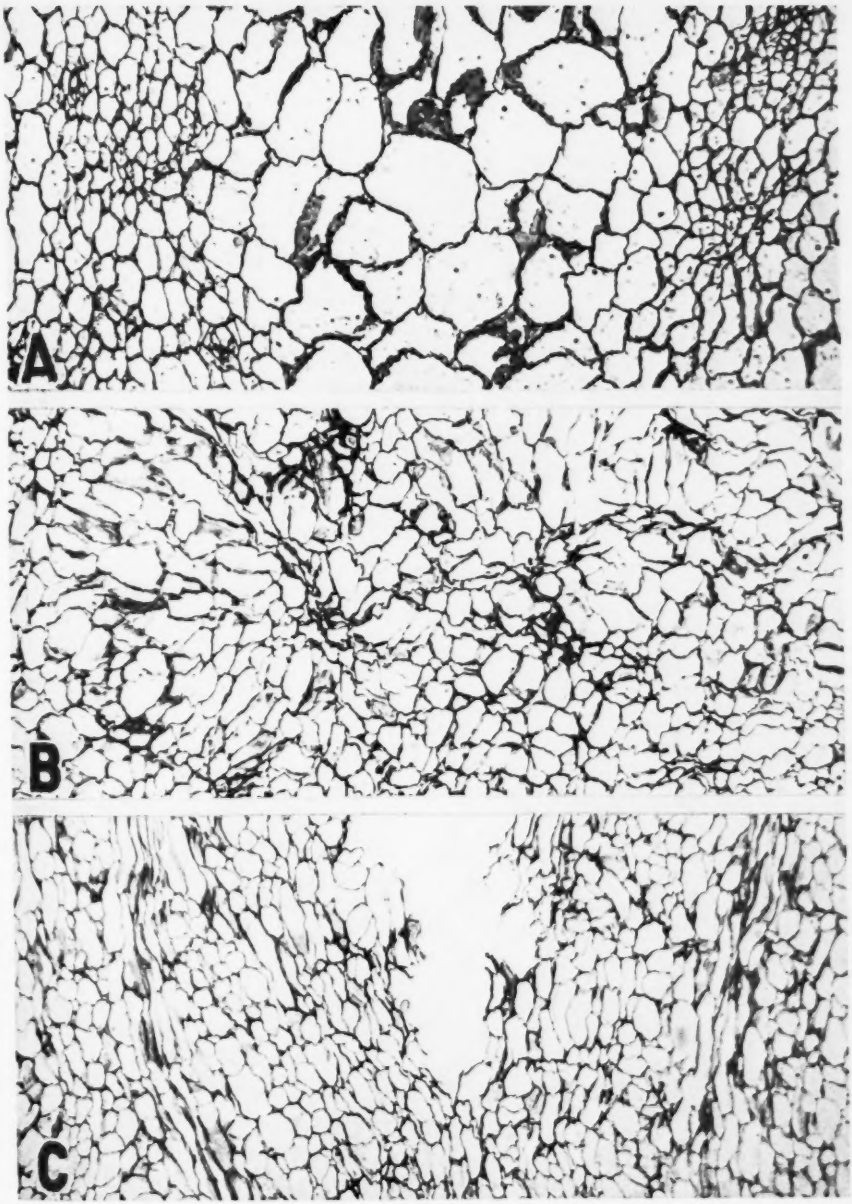

FIG. 3.—Section through hypocotyl in area between central portion of axis and cambium region: *A*, plus boron, showing vascular bundles and large xylem parenchyma cells; *B*, *C*, minus boron, showing absence of lignified vessels, disintegrated phloem cells, and smaller size of xylem parenchyma cells.

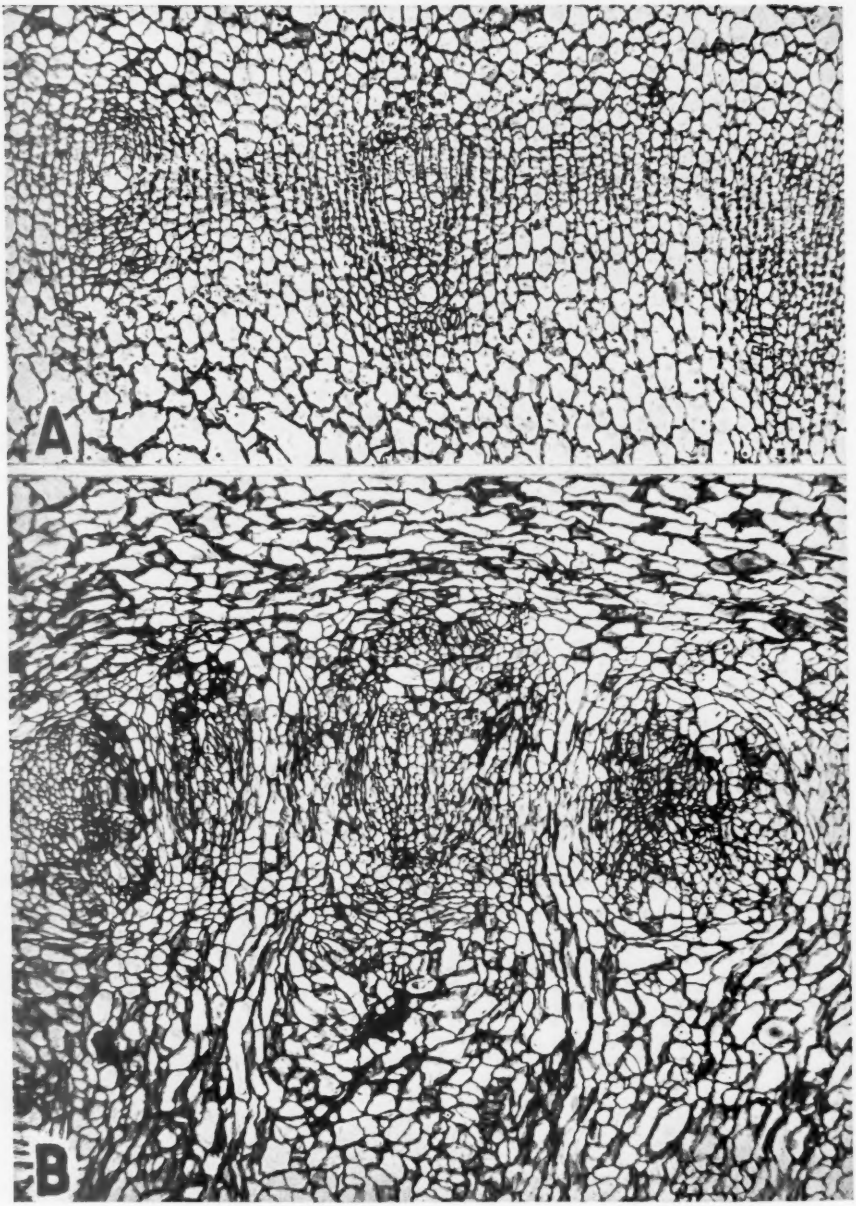

FIG. 4.—Cambium region: *A*, plus boron; *B*, unsplit portion of axis of minus boron plant showing dome arrangement of bundles and proliferation of xylem parenchyma and ray cells.

toms somewhat similar to those found in these experiments (root malformation), but they did not show any splitting. Their plants were grown in a boron-deficient soil, and possibly the deficiency was not so nearly complete. The plants of series 2, which received boron for the first 18 days, showed definite symptoms although less severe.

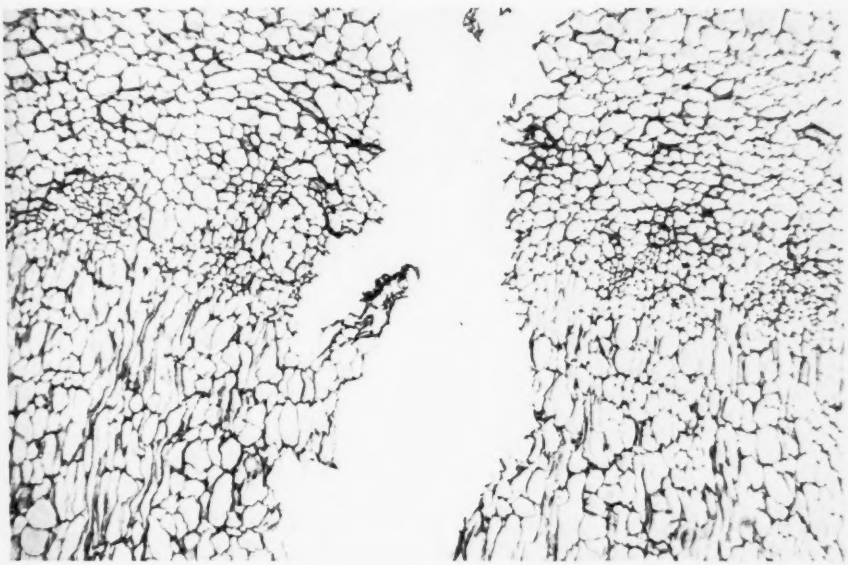

FIG. 5.—Cambium region of split portion of axis of minus boron plant showing indistinct vascular bundles, slightly lignified vessels, disintegrated phloem and cambium cells, and some proliferation of xylem parenchyma and ray cells.

B. EFFECT OF PHOTOPERIOD ON BORON-DEFICIENCY SYMPTOMS

Two experiments were conducted. In experiment 1 the seeds were planted May 17, 1940, and the plants harvested 42 days later. They were grown under plus and minus boron conditions; the minus boron plants received no boron throughout the experiment. One hundred and thirty plants were grown under each condition. Both boron series were grown under a short photoperiod of 7 hours (9:00 A.M. to 4:00 P.M.). Experiment 2 in this study was conducted as a check on the results of experiment 1 and on the boron-deficiency symptoms obtained in the former group of experiments, which were conducted under long photoperiod. In experiment 2 the plants were grown under the following four conditions: plus boron, long photoperiod; plus boron, short photoperiod; minus

boron, long photoperiod; minus boron, short photoperiod. The short photoperiod was also 7 hours, and the long photoperiod was the natural day length of that time (about 15 hours). The plants of experiment 2 were planted July 16, 1940, and were harvested 41 days later. Seventy plants were grown under each condition.

EXPERIMENT 1.—Under short photoperiod the boron-deficiency symptoms were not so pronounced as compared with the plus boron plants. The minus boron plants were slightly smaller and somewhat lighter in color. Their petioles were rather brittle and easily broken. The root-hypocotyl portions were smaller, more elongated, and a little lighter in color. Splitting occurred in only a few instances and in no case was so great as in the plants of the former experiments. Out of the total number of minus boron plants in this experiment, approximately 73 per cent were not split, 15 per cent had very slight splitting, and 12 per cent had more pronounced splitting (table 2).

TABLE 2

DATA OF EXPERIMENT 1, PART B. VOLUMES AND WEIGHTS REPRESENT AVERAGES PER PLANT ON 130-PLANT BASIS

GROWN UNDER 7-HOUR PHOTOPERIOD	VOLUME OF ROOT (ROOT AND HYPOCOTYL) (CC.)	WET WEIGHT		DRY WEIGHT		PERCENTAGE DRY WEIGHT			WET TOP-ROOT RATIO	DRY TOP-ROOT RATIO
		TOP (GM.)	ROOT (GM.)	TOP (GM.)	ROOT (GM.)	ENTIRE PLANT	TOPS	ROOTS		
Plus boron.....	4.61	7.24	4.56	0.52	0.25	6.51	7.20	5.40	1.59	2.12
Minus boron....	3.31	6.20	3.28	0.47	0.18	6.90	7.57	5.63	1.89	2.54

EXPERIMENT 2.—The plus and minus boron plants grown under long photoperiod responded similarly to those in series 1 and 3 in the study of gross boron-deficiency symptoms. Thirty days after the time of planting, the tops of the long photoperiod, minus boron plants were smaller and had narrower leaves than those receiving boron. The leaves had a bluish green color, very brittle petioles, and were rather stiff and curled. At this time there was scarcely any difference between the appearance of the top portions of the plus and minus plants grown under short photoperiod. The petioles of the minus boron plants, however, were somewhat stiffer. At the time of harvesting, the long-photoperiod, plus boron plants had well-developed large green leaves and underground portions 3.5–4 cm. in diameter and bright red in color. The leaves of the minus boron plants were smaller, bluish green in color, curled, and brittle. The young leaves were deformed and also brittle. The underground portions were light in color, long, and very slender, and all were cracked. They were 5–8 cm. long and 0.5–2 cm. in diameter. The leaves of the short-photoperiod, plus boron plants were lighter in color and

smaller than those of the long-photoperiod group. The underground portions were also considerably smaller, but well formed and bright red in color. The leaves of the short-photoperiod, minus boron plants were of about the same size and color as those of the plus boron plants, but they were also brittle. The underground portions varied greatly in size, but their average was not much below that of the plus boron group. They were lighter in color and somewhat deformed and had about the same percentage of cracking as similar plants of experiment 1. The data of table 3 show that on weight and volume bases the plus and minus boron plants grown under short photoperiod made about the same amount of growth, but both groups made less growth than the minus boron plants grown under long

TABLE 3

DATA OF EXPERIMENT 2, PART B. VOLUMES AND WEIGHTS REPRESENT AVERAGES PER PLANT ON 70-PLANT BASIS

CONDITION	VOLUME OF ROOT (ROOT AND HYPOCOTYL (CC.)	WET WEIGHT		DRY WEIGHT		PERCENTAGE DRY WEIGHT			WET TOP-ROOT RATIO	DRY TOP-ROOT RATIO
		TOP (GM.)	ROOT (GM.)	TOP (GM.)	ROOT (GM.)	ENTIRE PLANT	TOPS	ROOTS		
Plus boron, long photoperiod	12.37	19.28	11.68	1.14	0.55	5.43	5.90	4.67	1.65	2.09
Minus boron, long photoperiod	4.67	10.58	4.61	0.77	0.29	6.96	7.27	6.23	2.30	2.68
Plus boron, short photoperiod	3.55	9.75	3.52	0.62	0.18	6.00	6.33	5.07	2.77	3.46
Minus boron, short photoperiod	3.06	9.99	3.07	0.64	0.17	6.21	6.36	5.70	3.26	3.63

photoperiod. The long-photoperiod, minus boron plants exhibited the most severe deficiency symptoms. In terms of appearance and general condition, it is evident that the plus boron plants make relatively much greater growth under long photoperiod and the minus boron plants make more growth under short photoperiod. This is probably a matter of differential rate of growth, dependent on the photoperiod, with subsequent differential nutrient requirement. The long-photoperiod plants grow more vigorously and synthesize more materials and require more nutritive substances in order to do so. If an essential element is lacking, therefore, the deficiency symptoms in such plants are more severe than in less vigorously growing plants such as those in the short-photoperiod group. This is in agreement with EATON's (8) finding that the more vigorously growing plants exhibit more pronounced deficiency symptoms, and with those of WARINGTON (31) that the boron-deficiency symptoms of several plants were less pronounced under short than under long photoperiod.

Although the radish ordinarily flowers under long photoperiod (**10, 11**), only a few plants out of the entire numbers grown did so. At the same time other plants growing in the garden bloomed. This difference in the flowering response may be caused by difference in temperature or in light intensity. PLITT (**23**) found photothermal responses of the radish.

C. HISTOLOGICAL EFFECTS OF BORON DEFICIENCY

The root-hypocotyl portions of plus and minus boron plants were used in the histological study. The material was preserved in alcohol-acetic acid-formalin solution, dehydrated in an alcohol-tertiary butyl-alcohol series, imbedded, and cut at 7 μ. Samples were taken at 20 days after planting and every 4 days thereafter, until time of harvesting. Any differences shown in the plus and minus boron material in the early samples were more pronounced in later stages, and for that reason photomicrographs were taken only of material collected at the time of harvesting. Slides were made of material from two different experiments (plants grown a year apart), and the histological effect of boron deficiency was found to be similar in each case. The materials for the histological study were taken from plants grown under natural long photoperiod (about 15 hours).

A number of reports have shown that boron is important in the differentiation of the vascular system, and that in its absence there is general disintegration of the cambium and phloem. AGULHON (**1**) noted that boron is especially abundant in lignified tissue and bark, which indicated that it may have some function in the formation of vascular tissue or the compounds which impregnate these tissues. WARINGTON (**30**) found that boron deficiency in *Vicia faba* caused abnormal structure of both the stem and root, the chief features being: (*a*) hypertrophy of the cells of the cambium followed by degeneration with discoloration, or direct disintegration of the same tissue without previous enlargement; (*b*) frequent disintegration of phloem and ground parenchyma; and (*c*) slight development of xylem and in some cases ultimate disintegration of this tissue. BRENCHLEY and THORNTON (**5**) found that the vascular bundles related to the nodules on the roots of *Vicia faba* either failed to develop entirely or only to a small extent when boron was lacking. Anatomical studies on the tomato by JOHNSTON and DORE (**16, 17**) and by FISHER (**9**) showed disintegration of the phloem of the petioles and stems of boron-deficient plants. The total sugars and starch were more abundant in the leaves of the minus boron than in the plus boron plants. This was thought to be caused by injury to the conductive tissues, which reduced their capacity to translocate sugars. HAAS and KLOTZ (**13**) found boron to be essential for cell division in meristematic tissue of growing points such as buds, and equally essential for cambial activity. They found that the cambium and phloem disintegrated in minus boron citrus plants. The xylem was affected to a less extent. They also

found that sugar accumulated in the leaves of the affected plants because of the disintegrated conducting system, and that upon the addition of boron to these cultures there was rapid growth of new conductive tissue followed by decrease in the sugar content of the leaves.

CHANDLER (2) studied boron deficiency in eleven species of *Brassica*. In boron-deficient broccoli and Brussels sprouts he found that cell division stopped in the root tips and the root cap disintegrated. In rutabaga he observed crushing of meristematic cells of the stem tip, and in the root he found crushing of cortical cells, accumulation of cell inclusions in xylem parenchyma cells, elongation of certain cells near the cambium, and failure of the cork cambium to form cork. He also found crushing of cells in the cortex of the stem of cabbage, and when leaf blades of these boron-deficient plants were cut a cork cambium did not develop, but with similar treatment to plants supplied with boron, a cork cambium developed and healing occurred.

The effects of boron deficiency on the structure of the radish are somewhat similar to those in the preceding plants in that the vascular tissues are affected rather markedly. Sections through the hypocotyl of plus and minus boron plants show the general structure and the tissues affected by the deficiency (fig. 2). The vascular tissue near the center of the axis, including the primary tissue, was not affected. This is the first differentiated vascular tissue, and small amounts of boron may have been present from the seed or as impurities in the chemicals and sand, at the time of its maturation. After this amount of boron is exhausted the vascular tissue developing later would be lacking an element necessary for its development. Injurious effects on this later-forming vascular tissue again indicate that boron cannot be reutilized but that a continuous supply is necessary.

The vascular tissues occupying the area between the central, first formed, uninjured vascular tissue and the outer cambium region were markedly affected (fig. 3). Normally developed and lignified vessels in this area were entirely absent. The phloem cells disintegrated and left dead, dark-staining areas. The xylem parenchyma cells appeared to be uninjured and in some cases even rather active. The size of the xylem parenchyma cells of the boron-deficient radishes was generally smaller than those of the plus boron plants. Actual disintegration of these cells, as in the case of the ground parenchyma of *Vicia faba* reported by WARINGTON (30), was not observed.

The cambium region is of particular interest in that it shows a dual response (fig. 2). Axes which were cracked had very slightly developed vascular bundles in the cambium region near the cracks (fig. 5). Some disintegration of phloem also took place but not to the same extent as in the deeper areas. Well-developed vessels were almost entirely lacking, but some small, thin-walled vessels were

present. The cambium cells were almost entirely disintegrated. The xylem parenchyma, and particularly the ray cells, were rather active and showed proliferation.

The cambium region of axes which were not deeply cracked had an entirely different structure (fig. 4). The vascular bundles were fairly well developed and were arranged in pronounced domes and circular areas. A few vessels were present in these bundles, but they were rather thin walled, smaller, and much less lignified as compared with those of the plus boron sections. There was no pronounced phloem or cambium disintegration in these bundles, but the tissues were rather poorly developed and indistinct. Again the xylem parenchyma and particularly the ray cells were highly active. In some cases the proliferated ray cells formed complete rings around the bundles. Both these responses of the cambium region may be seen in the same axis, in which case the former is present at or near a crack and the latter where no splitting has taken place (fig. 2*C*).

These histological observations suggest that the splitting of the radish is a result of the failure of normal vascular tissue development, coupled with differential growth pressure. The lack of lignified xylem development and the areas of dead phloem create weak regions where the splitting may occur. The cessation of cambial growth, together with active xylem parenchyma and ray cells inside the cambium, result in internal pressure because of this unequal growth.

Summary

1. Radish plants deficient in boron produced small leaves with light bluish green color, which were (especially the petioles) brittle and curled down. The thickened underground portions were light in color, narrow and elongated, and severely cracked.

2. When boron was added up to 18 days after planting and then withheld, the top portion made greater growth but the brittleness of the leaves and the light color of the foliage were still apparent. The enlarged underground portion attained greater size and more normal shape but was also cracked.

3. Under short photoperiod the boron-deficiency symptoms were much less pronounced. Splitting of the underground portion was present in only about 27 per cent of the minus boron plants.

4. The vascular tissue near the center of the axis was not affected by boron deficiency.

5. Normally developed and lignified vessels in the area between the central, unaffected vascular tissue and the outer cambium region were entirely absent, and the phloem cells in this region had disintegrated.

6. The cambium region, if located at or near a crack, had slightly developed vascular bundles. The cambium and phloem cells were mostly disintegrated, and

well-developed vessels were lacking. The xylem parenchyma and ray cells were rather active.

7. The cambium region, if not located near a crack, had well-developed vascular bundles arranged in pronounced domes and circular areas. The vessels were unlignified, but there was no pronounced phloem or cambium disintegration. The xylem parenchyma and ray cells were here also highly active.

8. The splitting is caused by the failure of normal vascular tissue development, coupled with differential growth pressure.

The writer wishes to express his sincere thanks to members of the department of botany at the University of Chicago for their kind help and valuable suggestions made during the course of this work.

DEPARTMENT OF BOTANY
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WOOD STRUCTURE OF THUJA OCCIDENTALIS

M. W. BANNAN

(WITH FORTY-THREE FIGURES)

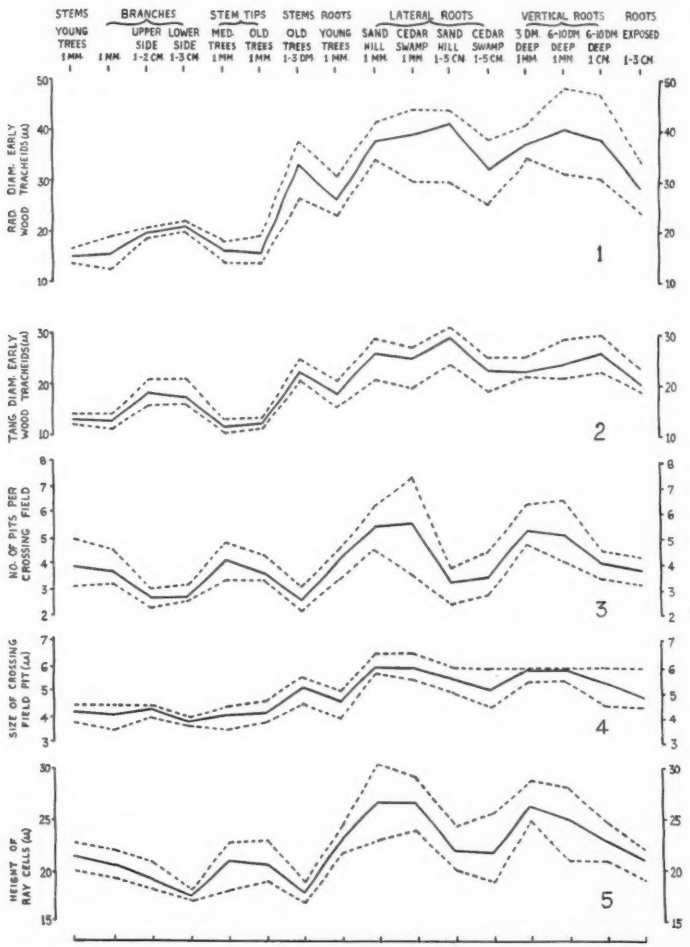
Introduction

Because of difficulties in obtaining sufficient amounts and variety of material, anatomical studies of coniferous woods have in many instances failed to provide adequate information for the positive identification of similar woods. Before criteria can be regarded as reliable, their constancy or range of variability within the species must be determined, an undertaking which—as BAILEY and FAULL (1) have pointed out—requires the examination of much material. In the present paper the results of an extensive study of the secondary xylem of the native white cedar, *Thuja occidentalis* L., are described.

All material was collected in southern Ontario within a 75-mile radius of Toronto. Some 230 wood samples were obtained from different parts of the tree, from trees of different sizes, and—in the case of roots—from different habitats and depths in the soil. The location in the tree, its size, and the type of habitat are indicated in figures 1–5. In these figures the term "stems young trees 1 mm." describes the inner wood 1 mm. from the pith in the stems of small trees approximately 0.5 m. tall. Data for the inner wood of branches, 1 mm. from the pith, were obtained from both the distal parts of old branches near the ground and from smaller branches at the tops of mature trees. Data for the outer wood of branches, 1–2 cm. from the pith on the upper side and 1–3 cm. from the pith on the lower side, were secured from the proximal portions of large branches of old trees. In the case of the "stem tips" the inner wood 1 mm. from the pith at the tops of the trees was studied, both of "medium" trees 3 m. tall and of "old" trees 10 m. or more tall. "Stems old trees 1–3 dm." describes the outer wood 1–3 dm. from the pith at the base of old stems.

In the case of roots the habit varied with the environment. The root systems in wet soils were flat and platelike, with all roots confined to the top layers of soil, whereas in well-drained sandy or gravelly soils there were in addition roots which extended deep into the ground. The latter are described as vertical roots to distinguish them from the widely spreading lateral roots located close to the surface. None of the roots included in the designation lateral were deeper than 6 inches, and the majority were only 2–3 inches deep. The lateral roots were collected from both wet and dry soils, "sand hill" indicating well-drained sandy soil on hilltops, and "cedar swamp," low-lying wet soils. Both the inner wood 1 mm.

from the primary xylem and the outer wood 1–5 cm. from the center were studied. All vertical roots were growing in sand or gravel, and the depths are shown



FIGS. 1–5.—Variation in different parts of tree: Fig. 1, radial diameter of tracheids in early wood of growth rings. Fig. 2, tangential diameter of tracheids. Fig. 3, number of pits per crossing field between tracheids and ray parenchyma cells. Fig. 4, size of pits in crossing field. Fig. 5, height of ray parenchyma cells.

by "3 dm. deep" and "6–10 dm. deep." Here again both inner and outer wood were examined. "Roots exposed" were lateral roots bared by soil erosion, and in these the data refer to the outer growth rings, 1–3 cm. from the center, laid down

after exposure. The various types of roots were examined at distances of 1–3 m. from the bole. All lateral, vertical, and exposed roots were growing from mature trees. A few roots of small trees 0.5 m. tall were studied and these are described in the figures as "roots young trees."

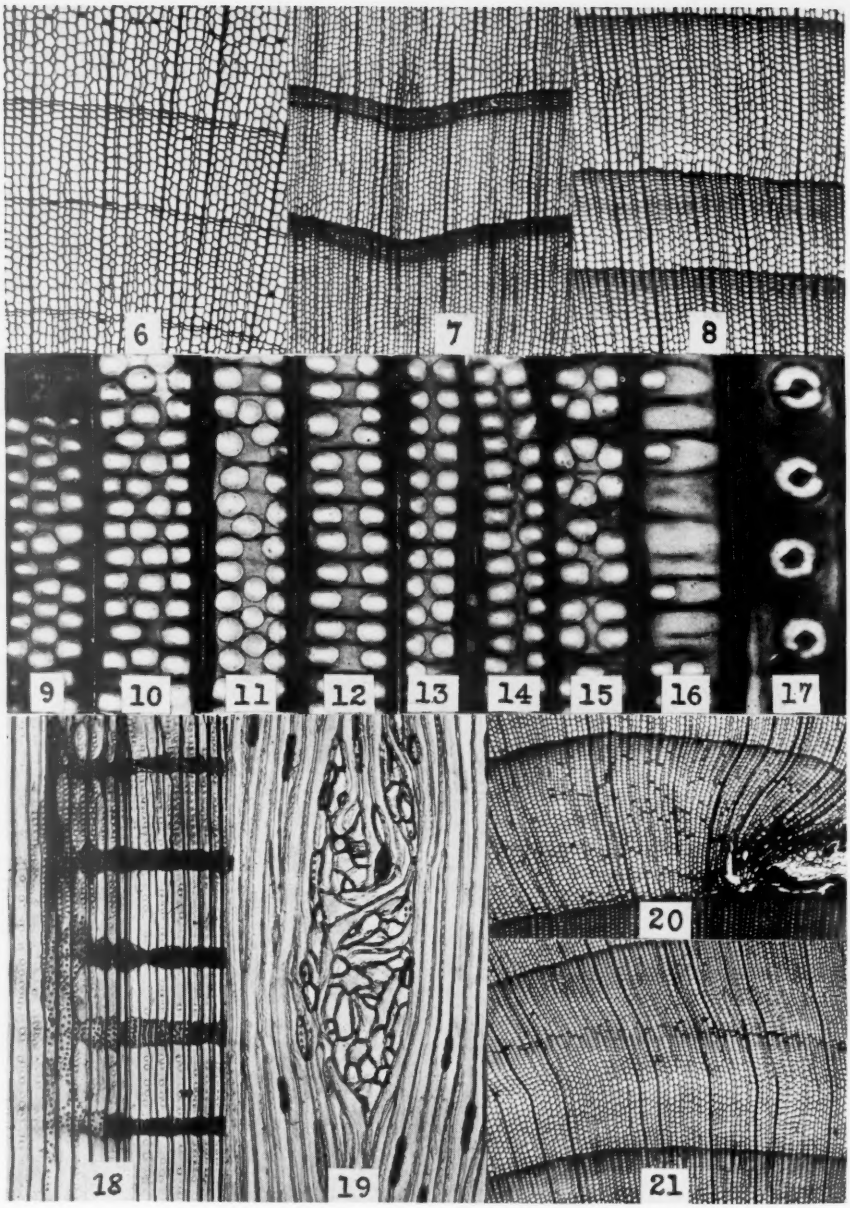
Usually more than ten specimens of each type of stem, branch, and root were examined for the compilation of the data in figures 1–5 and figures 22–25. In these figures the maximum average (in the specimen with the largest elements) is shown by the upper broken line, the grand average for all specimens by the middle solid line, and the minimum average (in the specimen with the smallest elements) by the lower broken line.

Observations

Tracheid size

Data on the radial and tangential diameters of tracheids in different parts of the tree are presented in figures 1 and 2. These values were determined from the early-wood tracheids in the growth rings and show the external dimensions of the cells from one middle lamella to the next. The measurements were made from transverse sections and included both wide and narrow cells. Had the determinations of the tangential diameters been made from only the central widest part of each tracheid, as observed in tangential sections, the values would have been 20–25 per cent higher than those in figure 2.

The smallest early-wood tracheids were observed in the inner wood of the stems of small trees, of branches both at the top and near the base of old trees, and of stem tips of both medium-sized and old trees. In the inner wood of these different parts of the tree the tracheids were uniformly small (figs. 1, 2). Outward from the center in both stems and branches the tracheids tended to increase in diameter. In roots the tracheids in the early wood of the inner growth rings were usually much larger than in stems and branches. As shown in figures 1 and 2, they were merely slightly larger in lateral than in vertical roots, and in the former only minor differences were noted between roots in dry and wet soils. This inner wood showed much greater variation among different roots of the same type or roots growing in the same habitat than among different kinds of roots. Outward from the center, however, distinctly different tendencies were noted. In the case of lateral roots growing in sand, the size of the tracheids increased slightly from the inner to the outer wood, whereas in roots growing in swampy soils the size decreased toward the outside. An even more noticeable decrease was observed in exposed roots, where the outer growth rings—laid down presumably after removal of the soil—were stemlike (fig. 7) and the tracheids were much smaller than in the inner wood (fig. 6). The amount of soil moisture did not appear to have had much effect on the texture of the inner wood of lateral roots, but, as already



FIGS. 6–21.—Fig. 6, open type of wood in lateral root. Fig. 7, conspicuous late wood in outer rings of exposed root. Fig. 8, stemlike wood in vertical root 6 dm. deep in soil. Figs. 9–16, various arrangements of pits on radial walls of tracheids in inner wood of lateral roots. Fig. 17, structure of torus. Fig. 18, radial view of newly formed rays in root wood. Fig. 19, tangential view of unusual tracheary ray in stem wood. Fig. 20, diffuse distribution of xylem parenchyma cells in stem wood. Fig. 21, tangential arrangement of parenchyma cells in stem wood. Figs. 6–8 ×38; figs. 9–16 ×360; fig. 17 ×850; fig. 18 ×65; fig. 19 ×110; and figs. 20, 21 ×38.

pointed out, differences were noted in the outer wood. The influence of site on tracheid caliber in stem wood has been dealt with by HARLOW (**6**).

In both stems and roots the tracheids in the late wood of the annual rings were much smaller than the early-wood tracheids. The transition ranged from abrupt (figs. 6, 7) to gradual (fig. 8), and the amount of late wood from the minimum of one row (fig. 6) to a considerable portion of the annual ring (fig. 8). In general the smallest amount of late wood occurred in the inner wood of lateral roots and the outermost wood of some old branches, whereas in the outer wood of roots growing in swamps, in the peripheral rings of exposed roots, in the outer wood of some vertical roots, and in the inner wood of most branches and stems, late wood was usually more prominent. There was much variation, however, among different specimens of the same type and often among different rings in the same specimen. For instance, the wood in vertical roots was highly variable, some specimens possessing an open type near the center and stemlike wood in the outer rings (fig. 8), while in others the wood was more or less uniformly intermediate.

The amount of the so-called compression wood, composed of rounded, thick-walled cells, likewise varied considerably. Sometimes the whole or greater part of growth rings in the inner wood of branches and stems was made up of such tracheids, while in other cases these were distributed in zones with sharp or gradual transitions to layers of thin-walled, rectangular tracheids between.

TRACHEID PITTING

The intertracheary bordered pits on the radial walls varied considerably in size and arrangement in different parts of the wood. They were usually largest in the outer rings of old stems and the inner wood of lateral roots, where they averaged 11–14 μ in diameter. In the annual rings the size tended to decrease from the early to the late wood. There was usually only one row of pits on the radial walls of the early-wood tracheids in the inner wood of stems and branches; usually one (but occasionally two) in the outer wood of old stems; and often two or sometimes three in the inner wood of lateral roots, where the tracheids were wider than elsewhere in the tree. When biseriate or triseriate, the pits were generally opposite (that is, in the same horizontal rows), but occasionally they alternated. Crassulae were exceedingly variable but on the whole were rather better developed in roots and the outer wood of old stems than in the inner wood of stems or branches. The greatest variation in the arrangement of pits and in the development of crassulae was found in the inner wood of lateral roots. Here the pits were usually biseriate and opposite, with conspicuous straight crassulae (fig. 12), or grouped with curved crassulae (fig. 15); but sometimes the crassulae were indistinct or absent (fig. 13), and in a few cases the pits alternated and crassulae were lacking (fig. 14). When triseriate, the pits were generally opposite, with or without crassu-

lae (fig. 10), less often mixed, sometimes with straight crassulae (fig. 11), and occasionally alternate, generally without crassulae (fig. 9). Toward the overlapping ends of the tracheids the pits tended to be crowded, but elsewhere were often more widely spaced (fig. 16). The pit apertures were usually broadly oval or round in the early wood and slitlike in the late wood.

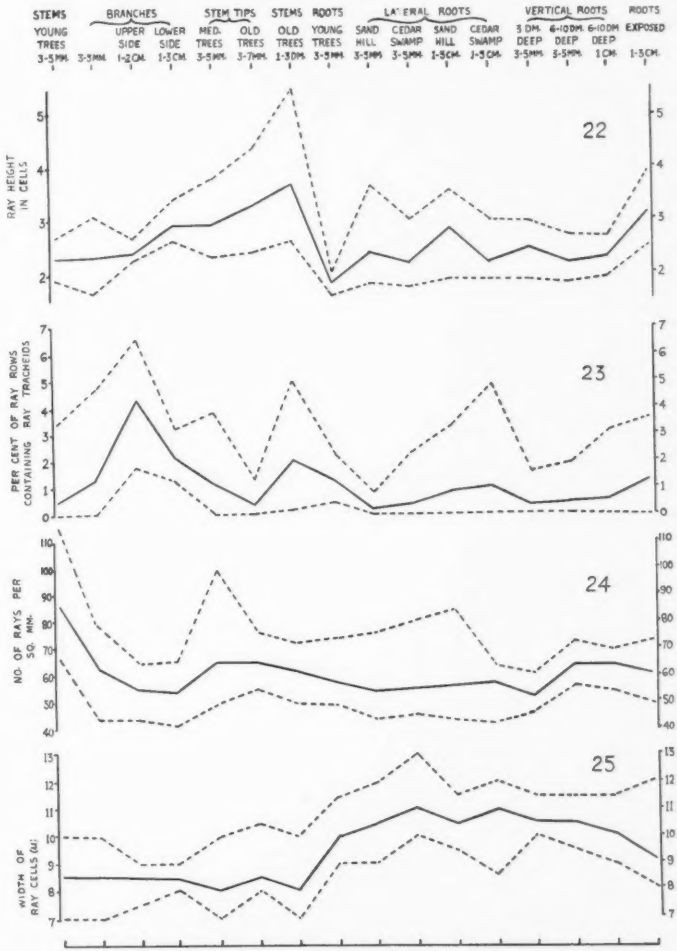
As shown in figures 9–16, tori were not discernible in most of the intertracheary pits. Only sporadically in different parts of the tree were tori recognized. They ranged in size from one-half the diameter to slightly larger than the pit aperture, and had irregular or lacerate margins (fig. 17). The absence or feeble development of the torus was a striking feature of the wood.

Bordered pits in the tangential walls of the tracheids occurred characteristically in the late wood and only rarely in the early wood of the growth rings. In the latter case they were associated with injuries or abnormalities. For example, in two specimens of stem wood certain of the radial rows of tracheids in the early wood of one of the growth rings were composed of cells which were much narrower radially than the typical tracheids nearby. These particular cells resembled late wood tracheids in size and shape, and—like the latter—had pits on their tangential walls. The pits were sporadic, however, and approached in size the pits on radial walls. Tangential pitting also occurred in early-wood tracheids in or near injuries. The usual tangential pits in the late wood varied greatly in size, but generally averaged 5–7 μ in stems and branches and 7–9 μ in roots.

The number of pits per crossing field between tracheids and ray parenchyma cells ranged from none to more than fifteen, depending upon size and shape of the ray cell, size of the tracheid, location in the annual ring, and part of the tree. There were, for instance, more pits between tracheids and the vertically elongated or squarish-shaped cells in the first-formed parts of new rays (fig. 18) than between tracheids and the lower, radially elongated cells in the succeeding parts of the rays. A comparison of figures 1, 3, and 5 shows that the most numerous pits occurred in the inner wood of roots, where the tracheids were wider and the ray cells higher than in other parts of the tree, and fewest in the outer wood of old stems and branches, where the height of the ray cells was at a minimum. Outward from the center in stems, branches, and roots the number of pits tended to decrease, as did also the average height of the ray cells. The range in different parts of the tree was considerable, from an average of 5.6 in the inner wood of lateral roots to only 2.6 in the outer wood of old stems. The values shown in figure 3 were determined from rays two or more cells high consisting entirely of radially elongated cells and refer only to the early wood. In each growth ring the number decreased toward the late wood.

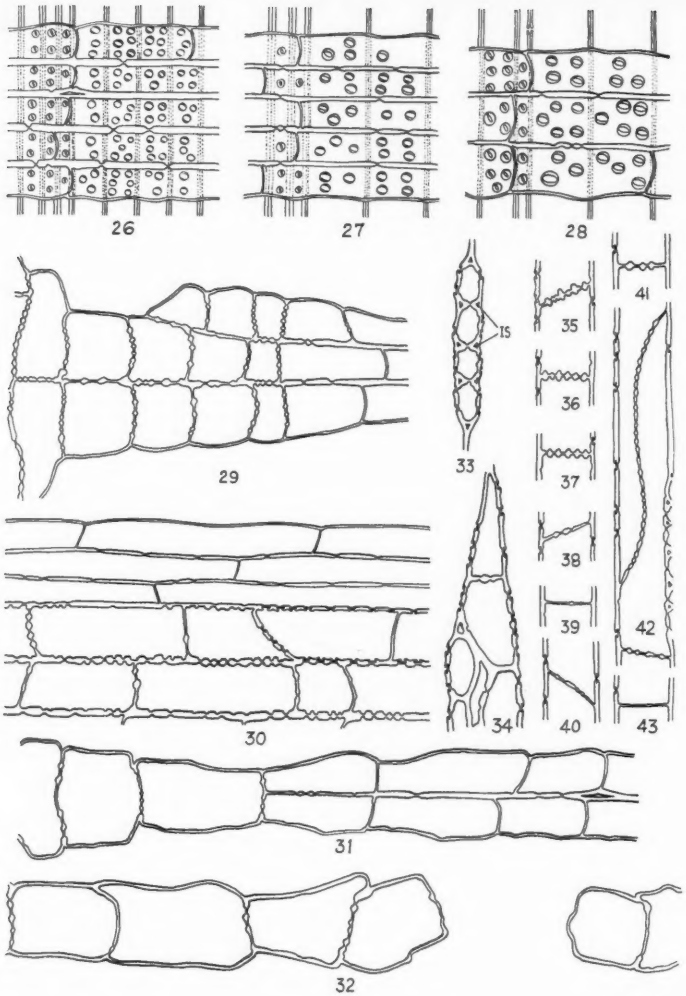
The pit apertures in the crossing fields of the early-wood tracheids were exceedingly variable, ranging from narrowly elliptic with broad borders to round

with reduced or absent borders. Generally the pits with the largest apertures and narrowest borders were found in the inner wood of stems and branches (fig. 26),



FIGS. 22–25.—Variation in different parts of tree: Fig. 22, ray height in cells. Fig. 23, percentage of ray rows containing ray tracheids as determined from radial sections. Fig. 24, number of rays per sq. mm. of tangential section. Fig. 25, width of ray cells in tangential section.

the outer wood of some stems (fig. 27), and the outer wood of certain roots such as those exposed on the surface of the ground. Pits with narrower apertures were observed most often in the inner wood of roots (fig. 28), but in some cases directly



FIGS. 26–43.—Fig. 26, ray structure in inner stem wood. Fig. 27, same in outer wood of old stem. Fig. 28, ray structure in lateral root. Fig. 29, wall structure of cells in ray beginning at pith. Fig. 30, same of cells in unusual ray in old stem wood. Fig. 31, same of cells in ray arising at center in lateral root. Fig. 32, same of cells in newly formed ray in old stem wood. Fig. 33, tangential view of ordinary ray in stem wood showing intercellular spaces (*is*). Fig. 34, tangential view of unusual ray in branch wood showing lack of intercellular spaces. Figs. 35–43, tangential views showing structure of transverse walls of xylem parenchyma cells in outer wood of old stems.

opposite dispositions were found. The orientation of the apertures varied from horizontal to a definite inclination. In the late-wood tracheids of growth rings throughout the tree the apertures of the crossing-field pits were generally slitlike and steeply oriented.

The size of the crossing-field pits varied considerably, even within the same field, but in the early wood averaged about 5–6 μ in the inner wood of lateral roots, 5 μ in the outer wood of old stems, and 4 μ in the inner wood of branches and stems (fig. 4). In general the size tended to increase outward from the center in stems and to decrease outward from the center in roots, and from the early to the late wood in growth rings throughout the tree.

Xylem parenchyma

Xylem parenchyma cells were variable in distribution. When abundant, as for instance in the vicinity of injuries, they were diffuse (fig. 20) or more or less aggregated in tangential arrangement (fig. 21). When scarce the cells were widely dispersed, sometimes only in late wood, less often in the early wood. Generally xylem parenchyma cells were more abundant in the inner wood, particularly of branches and roots, than in the outer rings.

The transverse walls of the parenchyma cells were occasionally only slightly thickened (figs. 39, 40, 43), but more often were definitely and regularly thickened or beaded (figs. 37, 41) or irregularly thickened (figs. 35, 36). The walls, while usually at right angles to the long axis of the cell (figs. 36, 37, 39, 43), were sometimes oblique (figs. 35, 38, 40), and rarely were almost vertical (fig. 42). Often the transverse walls were somewhat thinner in late than in the early wood of the growth rings, but otherwise no trends were apparent in the amount of wall thickening in different parts of the tree.

Strand tracheids

In the material studied these cells were found only in the vicinity of injuries. The elements were made up partly of parenchyma, partly of tracheary cells.

Size and distribution of rays

The majority of the rays were uniseriate, seldom more and usually much less than 1 per cent of the rays being partially biseriate or wider. Their height ranged from one to more than twenty cells. In stems and branches the height tended, on the average, to increase from the inner to the outer wood, attaining a maximum in the outer rings of old stems (fig. 22). There was considerable variation among different specimens, the average in the periphery of old trees ranging from 2.7 cells in one specimen to 5.5 cells in another. In most roots the rays were relatively low, the average in different specimens fluctuating between 1.7 and 3.7

cells, with the grand average ranging between 2.2 and 2.8 cells (fig. 22). The height tended to increase in the outer wood of exposed roots, where the average was 3.1 cells. All these determinations were made from radial sections.

The size and shape of the individual ray cells were exceedingly variable. The cells in the first-formed parts of new rays, particularly those arising in the inner wood, were often vertically elongated (fig. 18), whereas most of the cells in the succeeding parts were lower and lengthened in the radial direction. The length of the latter was greater in the early than in the late wood of each annual ring, and was usually greater in the outer wood of old stems than in the inner wood. The average height of these radially elongated cells fluctuated considerably in different specimens, but was generally higher in roots than in stems or branches (fig. 5), and in both roots and stems tended to decrease outward from the center. In lateral roots the average height decreased from 27 μ near the center to 22 μ in the outer wood and in stems declined from 22 μ near the pith to 18 μ in the periphery. The average width of the ray cells was 10–11 μ in roots and approximately 8 μ in stems and branches (fig. 25).

It has been shown by ESSNER (**4**) for several conifers that the number of rays in the stem tends to decrease outward from the center, but, as SHIMAKURA (**9**) has demonstrated, this tendency may be modified by the width of the succeeding growth rings. In the material of *Thuja occidentalis* examined by the writer the average number of rays dropped from 87 per sq. mm. in the inner wood to 61 in the outer wood of old stems (fig. 24), and in branches decreased from 63 near the center to 55 and 53 in the outer rings. There was also a decrease from the base to the upper levels in the stem. At the same distance from the pith there were 87 rays per sq. mm. in the stem bases of small trees as compared with an average of 65 per sq. mm. in the stem tips of trees 3 m. and 10 m. or more tall. JACCARD (**7**) has described similar distributions in other conifers. In roots no particular tendencies in distribution were noted. The rays as a rule were of approximately similar distribution in both inner and outer wood (fig. 24). Much greater variation was observed among individual roots of the same kind than among different parts of the root or among roots in different habitats or depths in the soil. In this lack of correlation *T. occidentalis* differed markedly from certain other conifers (**2**).

In addition to the ordinary uniseriate rays just described there were sporadic rays which differed strikingly in size, structure, and distribution. These unusual rays were sometimes much higher than the ordinary ones; their width ranged from one to occasionally ten or more cells; the individual ray cells were of many diverse shapes; and in some instances parts of the ray were largely tracheary (fig. 19). They originated from uniseriate parenchymatous rays usually traceable to the pith or primary xylem, and toward the outside broadened and subsequently

followed different courses of development. Some connected with adventitious roots, others split up into smaller rays, and yet others became gradually reduced in size so as to resemble ordinary rays again. They were found throughout the aerial parts of the tree, but their distribution was erratic (3). The most frequent occurrence was observed in the outer wood on the lower side of large branches, where in one instance they attained an average frequency of 65 to the sq. cm. (as compared with 5300 ordinary rays per sq. cm.). They were seldom found in roots.

Ray structure

The ordinary uniseriate rays which began at the pith or in the early secondary xylem were usually parenchymatous throughout. Rays of later origin, particularly those arising in the outer wood of stems and branches, generally contained ray tracheids in their first-formed parts; but in the succeeding parts, where the ray height increased, the cells became parenchymatous. Sometimes such recently formed rays containing ray tracheids were applied to the margins of older parenchymatous rays and formed marginal tracheary rows along the latter.

Because ray tracheids were usually restricted to new rays or marginal rows formed as already described, they did not constitute a large proportion of the tissue in the ordinary uniseriate rays. The most frequent occurrence was in the outer wood of branches and stems (fig. 23), where on the average 2–4 per cent of the ray rows contained ray tracheids, but even here their distribution was erratic. For instance, in old stems the range was from nearly zero in one specimen to more than 5 per cent in another. Moreover, these rows were not exclusively tracheary but in some cases contained only some ray tracheids intermixed with parenchyma cells, so that the actual proportion of ray tracheids in terms of parenchyma cells was even less than indicated in figure 23. These determinations were made from radial sections where ray tracheids were more obvious than in tangential sections. In roots ray tracheids were scarce. Their most frequent occurrence was in the outer wood of certain exposed roots and of roots growing in swamps, where the wood was stemlike in other features, but even here the average was only about 1 per cent (fig. 23). In the inner wood ray tracheids were yet more infrequent. A noteworthy feature of the distribution of ray tracheids in both roots and stems was the wide variation among different specimens of the same type.

The ray tracheids were of many diverse shapes, especially in the earliest-formed parts of the new rays; but farther along in the ray, or where the new rays were applied to the margins of older ones, the cells tended to be elongated radially. The bordered pits on the radial walls adjoining tracheids were generally relatively large, averaging about 8 μ, but in some instances graded down to 3 μ. Some ray tracheids had only large pits; others exhibited mixtures of large and small pits; and a few, particularly those located nearest the point of ray origin, sometimes

possessed only small pits. Occasionally the pits were only dimly outlined and the walls were thin, the appearance suggesting that the protoplasm had disappeared before wall thickening had been completed. Bordered pits on the horizontal walls in contact with ray parenchyma cells ranged from few to numerous, and the number of pits on the tangential walls between ray tracheids also varied considerably. Sometimes these walls lacked pits.

The preceding descriptions apply only to ray tracheids in the ordinary uniseriate rays. In addition to the latter, however, there were sporadic unusual rays which differed markedly in size and structure. They originated from uniseriate parenchymatous rays but subsequently underwent strikingly different courses of development (3). The tracheary cells in these unusual rays were highly variable in number, position in ray, shape, size, and structure. They fluctuated from few—sometimes interspersed among thick-walled parenchyma cells in the central portions of the ray (fig. 30)—to many, comprising the bulk of the ray (fig. 19). Their shape ranged from radially elongated to rectangular, squarish, vertically elongated, and varied angular forms (fig. 19). Where the unusual rays were only one or two cells wide and ray tracheids were appearing for the first time, these cells were often radially elongated or somewhat squarish in shape, and resembled typical ray tracheids with bordered pits commonly on all walls but sometimes absent from tangential ones. As the unusual rays widened these cells were sometimes followed by others in which there was progressive lengthening in the vertical direction, so that some of the tracheary cells came to resemble short fusiform elements. As in the vertical tracheids comprising the wood, the bordered pits on the radial walls of these cells were often large, occasionally attaining the size of intertracheary pits, and faint crassulae were discernible above and below. The tangential walls often lacked pits. Where such series of transitional types occurred it seemed clear that certain of the ray initials had elongated until they acquired the stature of short fusiform initials. In some cases this continued enlargement resulted in a splitting up of the ray into separate smaller units, a process which sometimes was aided also by penetration of adjoining ordinary fusiform initials into the ray. In other instances the ray initials, after having elongated, gradually shortened, and some ceased dividing so that the ray tissue was reduced in quantity. Other unusual rays, instead of becoming subdivided into smaller ones, connected with adventitious roots, the root meristems apparently having been organized in the phloem or cambial portions of the rays. In the wide parts of these rays, where the vascular tissues of the newly formed root were first appearing, the tracheary cells showed gradations in size, shape, and structure from more or less typical ray tracheids in the ray proper to primary xylem tracheids in the new root.

Ray parenchyma cells in the ordinary uniseriate rays typically had thin tangential walls and thick horizontal and radial walls (figs. 26–28). The latter were

described by BAILEY and FAULL (1) as thickened primary walls bearing deeply depressed primary pit-fields. The tangential walls were usually thin (figs. 26–28) but sometimes were definitely thickened, particularly near the pith (fig. 29), less often near the center of roots (fig. 31), and rarely in the first-formed parts of new rays arising in the outer wood (fig. 32). The horizontal walls were generally thick, with widely spaced pit-fields (figs. 26–28), but sometimes were comparatively thin, especially in new rays arising near the center of roots. As a rule the horizontal walls of the cells in the older parts of the rays were slightly thinner in the outer wood of old stems and the outer wood of some roots than elsewhere in the tree. Indentures, or recessions in the horizontal wall adjoining the point of contact with the tangential wall, have been described by PEIRCE (8) as regularly present and pronounced in *Thuja*, but in material examined by the writer were variable. They were usually present in the outer layers of old stems but occasionally were shallow or absent; they were often well developed in the inner wood of stems and branches but at other times were not discernible; and in the inner wood of some lateral roots they were frequently lacking or only feebly developed.

The ray parenchyma cells usually retained their protoplasm for several years, but in some cases, especially in the first-formed parts of rays or where a row in an old ray had ceased, the protoplasm disappeared early. The walls of these cells were often thinner than in the living cells, and the number of pits in the crossing field was occasionally noticeably reduced. In one or two instances only an outline or "ghost" was discernible, and the adjoining tracheids lacked pits in the crossing field.

Yet other types of parenchyma cells were observed, particularly in some of the sporadic unusual rays. Here certain of the cells, as shown in the lower two rows in figure 30, had thick lignified walls with numerous pit-fields. The thickness of the tangential walls especially was in marked contrast to the uniformly thin condition in the corresponding walls of the more typical ray cells in the upper three rows. The protoplasts of the thick-walled cells disappeared early in some instances but in others remained as long as in the typical ray parenchyma cells. Intercellular spaces between the thick-walled cells and the adjoining tracheids were generally much reduced or absent (fig. 34), while those associated with typical ray cells were conspicuous (fig. 33). Where the unusual rays attained their maximum width the range of structural variability in the parenchyma cells was even greater. From cells with thick, knoblike, heavily lignified walls there were gradations to cells with uniformly thin, unlignified or slightly lignified walls on all sides. Some of the cells were dead; others living. The cell shape ranged from round and distended to irregular and angled, and occasionally some of the thin-walled cells had collapsed. The diverse types and shapes of cells occurred in various proportions in different rays and in different parts of the same ray. Intercellular spaces were variable.

Discussion

Some features of the wood of *Thuja occidentalis* varied considerably from one specimen to another without evidence of definite trends in any parts of the tree. In other features distinct tendencies were apparent in some parts of the tree though perhaps not in other parts. For instance, the tracheids increased in size outward from the center in stems but not in roots, and as a rule were noticeably larger in roots than in stems; the number of rays decreased outward from the pith in stems but in roots remained relatively constant; the ray height increased outward from the center in stems and in certain types of roots, and in the inner wood was approximately the same in roots as in stems or branches; the height of the individual ray cells and the number of pits per crossing field decreased toward the outside in both stems and roots. Certain of these tendencies have been noted in other genera, and they may prove to be general among the conifers. For example, the decrease in number of rays outward from the pith has been noted in several genera (**4**, **5**). In other cases, however, the degree or even direction of trend varies with the genus. It has been shown in *T. occidentalis* that the average ray height in the inner wood of root and stem is similar, whereas in some of the Abietineae the rays in certain types of roots are definitely higher than in stems of like size (**2**). Preliminary investigations have indicated that divergency in trend may occur even among the Cupressineae. Hence it is unwise to assume that tendencies in anatomical variation noted in one genus necessarily occur in another. Variability in structure and divergency in trend render imperative the use of homologous parts of the tree when comparing or contrasting the woods of different conifers.

Although many specimens of the various other Cupressineae were studied, neither sufficient quantities nor variety of material were available to allow satisfactory comparisons with *T. occidentalis*. For this reason no attempt will be made to select characters useful for the identification of *Thuja* wood or its separation from other Cupressineae. PEIRCE (**8**), in his descriptions of *Thuja*, referred to the large size of the apertures in the crossing-field pits as being unique, and described indentures in the horizontal walls of the ray cells as "regularly present and pronounced." Both these features were often well developed in stems but in some specimens and in other parts of the tree were variable, the indentures particularly being too inconstant to be diagnostic. SLYPER (**10**) in his key separated *T. occidentalis* from *T. gigantea* and *T. standishii* on the absence of thickenings on the transverse walls of the xylem parenchyma cells. He stated that the walls in *T. occidentalis* were nearly smooth, whereas the writer found them to be sometimes smooth but more often definitely thickened or beaded. Because of such variability, attempts to differentiate closely similar woods by utilizing only single anatomical characteristics of the type here mentioned seem futile. If identifications are to be positive, not single but rather combinations of characters must be used.

Summary

1. Studies of samples from different parts of the tree, from trees of different sizes, and—in the case of roots—from roots growing under different environmental conditions, revealed much variation in the structure of the secondary xylem.

2. Some features usually recognized as variable, namely, size of tracheids, number and size of crossing-field pits, height and distribution of rays, and height and width of ray cells, were found to vary widely; but in some cases definite trends were recognizable in different parts of the tree.

3. Other characters more generally regarded as diagnostic, such as the arrangement of the intertracheary pits on the radial walls of tracheids, the degree of development of the torus, the presence or absence of crassulae, the size and shape of the apertures in the crossing-field pits, and the thickness of the walls of ray and wood parenchyma cells, also varied greatly, often within the same piece of wood.

4. In view of such variability it is obvious that caution must be exercised in the selection of diagnostic characters for the identification of woods.

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GEMMAE-CUP PRODUCTION IN MARCHANTIA POLYMORPHA AND ITS RESPONSE TO CALCIUM DEFICIENCY AND SUPPLY OF OTHER NUTRIENTS[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY **532**

PAUL D. VOTH

(WITH SIX FIGURES)

Introduction

Excellent adaptability to nutrient supply and photoperiod has been reported recently for *Marchantia polymorpha* L. (**15**). Nutrient triangles with relatively small numbers of positions were adequate to effect distinctive growth responses when calcium, phosphate, and nitrate supply was systematically varied. The present study deals principally with the effect of relatively smaller proportions of the six common ions, with particular reference to lower limits of calcium and phosphate supply and to larger concentrations of nitrates. This is accomplished by employing triangles each having fifty-five positions. Similar nutrient triangles have been employed by SCHREINER and SKINNER (**11**), TRUE (**14**), and others.

As previously, records were made of changes in gross appearances of the plants, increases in total area, accumulation of dry weight, and formation of gemmae cups. From the data secured it is possible to suggest the relative proportions and concentrations of the commonly used nutrient salts conducive to the greatest vegetative development of this plant. The anatomical details of the apices of plants growing in nutrient solutions deficient in calcium may give clues to the function of calcium in meristematic and differentiating cells and tissues. Comparison of the total number of gemmae cups on antheridial and archegonial thalli seems to offer possibilities in the identification of the sex of vegetative plants of *M. polymorpha*.

General methods

The strains of plants used in this investigation are from the clones used in experiments 8 and 9 (**15**) and originated as follows:

Culture A: One male plant selected from plants growing in the greenhouses of the University of Chicago, May 16, 1939.
Culture B: One female plant similarly selected, May 15, 1939.

From the new tips which form dichotomously as the plant grows, these clones have been perpetuated uninterruptedly.

[1] This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

All experimental work was conducted in the greenhouse, using the culture method of placing six plants on a disk of glass cloth supported by a glass rack and placed in the open half of a moist chamber. Each culture was supplied with about 200 cc. of fresh solution daily (**15**). The plants and apparatus received a daily pressure-rinsing with distilled water, which also served to remove excess algae from the surface of the glass cloth and from the water line in the moist chamber.

All plants grew for 32 days, September 24 to October 27, 1940. Supplementary light was supplied with 200-watt Mazda filament bulbs in reflectors, suspended about 33 inches above the plants. A maximum of 200 foot-candles directly underneath the reflectors and more than 100 foot-candles between the lights at the level of the table top was recorded at the beginning of the experiment, when reflection from the glass cloth was maximum and plant size minimum. These lights were on from sundown until 2:00 A.M., a total photoperiod of 18 hours in each 24-hour period.

Because of experience gained previously, the phosphate supply was considerably decreased, the nitrate supply was increased, and the other ions were given in relatively the same proportions as in experiments 8 and 9. The molar concentration of each ion as well as the amount of each salt used is shown in table 1.

Of the fifty-five units constituting each triangle, only thirty-seven were actually used in the present study. Since the omission of a single ion resulted in striking symptoms in previous experiments (particularly K, Ca, NO_3, and PO_4), many positions along the sides of the triangles were not repeated. The positions actually employed in this study are indicated by squares and the unused ones by circles in figure 1. In a similar triangle, anions were varied and cations remained constant; the NO_3 position was at the top, PO_4 at the lower left, and SO_4 at the lower right.

Male and female plants of cultures A and B, respectively, were grown in separate moist chambers, but the two cultures were always adjacent to facilitate changing of solutions and to provide as comparable a habitat as possible for both sexes.

As in experiments 8 and 9, gemmae cups were counted, all plants were photographed by groups, areas were determined photometrically, fresh weights were recorded, one plant from each dish was preserved in formalin–acetic acid–alcohol preservative, and the remaining plants were weighed when dry. The percentage dry weight of five plants was used to calculate the dry weight of six plants. The present investigation is designated experiment 10.

Investigation

Response to omission of ions

Cation triangle.—Each *Marchantia* cutting, when planted on September 24, averaged 3 sq. cm. in area. Plants on positions 10, 28, 1, and 55 received no calcium, and the latter two were given no magnesium and no potassium, respectively. These cultures grew only slightly, with a maximum final area of only about three

TABLE 1

Concentration of salts in the six stock nutrient solutions used in making up 74 other combinations. To each solution was added 0.2 p.p.m. of $MnSO_4$, $ZnCl_2$, and $Na_2B_4O_7$, and also 0.02 p.p.m. of $FeSO_4$. The nine salts are identical with those of experiments 8 and 9 (15)

Molar concentration	Salt content (cc. of 0.5M solution) per l.	Molar concentration	Salt content (per liter)	Molar concentration	Salt content (per liter)
Cations varied					
K solution		Ca solution		Mg solution	
K....0.0054	.....	Ca....0.0027	.....	Mg....0.0029	.....
NO_3....0.0032	6.40*	NO_3....0.0032	3.20 cc. of 0.5M	NO_3....0.0032	3.20 cc. of 0.5M
PO_4....0.0004	0.80†	PO_4....0.0004	0.0504 gm.‡	PO_4....0.0004	0.0698 gm.§
SO_4....0.0009	1.80	SO_4....0.0009	0.1550 gm.	SO_4....0.0009	1.80 cc. of 0.5M
Anions varied					
NO_3 solution		PO_4 solution		SO_4 solution	
NO_3....0.0051	.....	PO_4....0.0042	.....	SO_4....0.00255	.....
K.....0.0009	1.80*	K.....0.0009	1.80 cc. of 0.5M†	K.....0.0009	0.90 cc. of 0.5M
Ca.....0.0012	2.40	Ca.....0.0012	0.3026 gm.‡	Ca.....0.0012	0.2066 gm.
Mg.....0.0009	1.80	Mg....0.0009	0.1570 gm.§	Mg....0.0009	1.80 cc. of 0.5M

* As KNO_3. † As KH_2PO_4 ‡ As $Ca(H_2PO_4)_2 \cdot H_2O$. § As $MgHPO_4 \cdot 3H_2O$, etc.

times the original. A smaller final area than the original may be attributed to disintegration of the dead plants (solution 55). After 4–6 days all plants in cultures deprived of calcium became black tipped and finally died, except a few which regenerated new thalli from the ventral surface of the older plant. Plants on solution 55, lacking calcium and potassium, blackened soonest and were smallest in area.

When potassium is omitted from nutrient solutions, as in positions 49, 52, 46, and 55 (with the latter two also lacking magnesium and calcium, respectively), the growth responses are as follows: Plants on solution 55 respond to the omis-

sion of calcium after a few days. After 18 days, position 46 still possesses small plants (fig. 2), but the characteristic tan color of the older thallus parts—especially along the margins of the wings near the tip—indicates a lack of potassium.

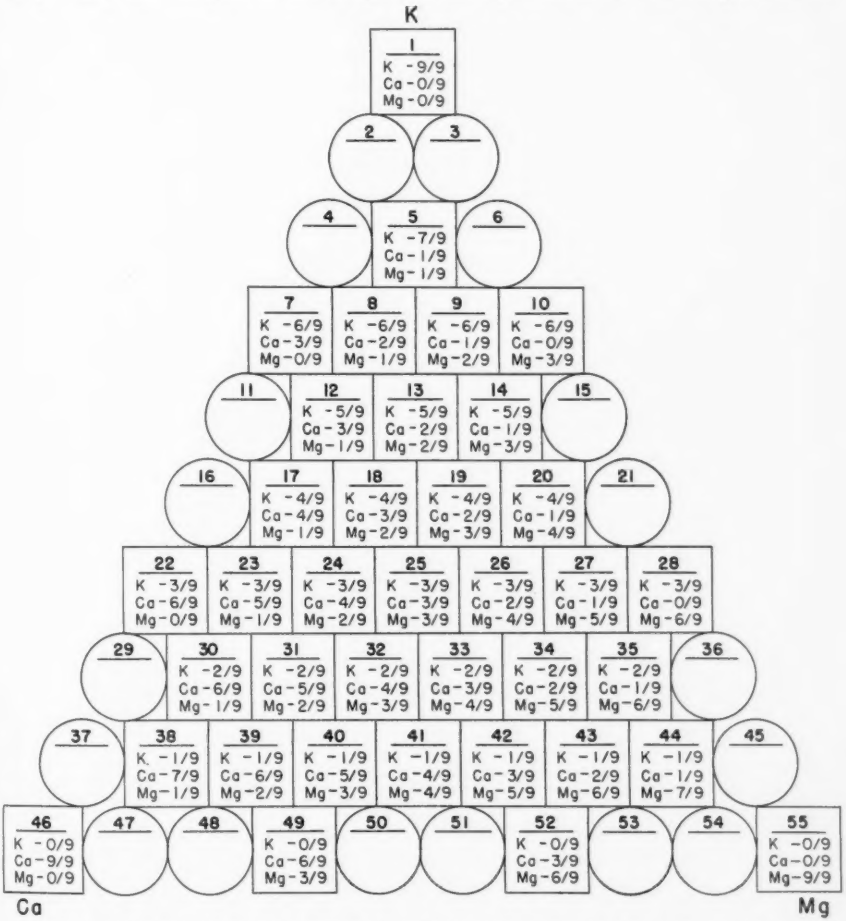

FIG. 1.—Cation triangle showing method of combining stock solutions, on basis of ninths, to secure a range of fifty-five solutions varying in cations and constant for anions. Each apical position lacks two cations; each side of the triangle, one; and the center is supplied with all three in varying proportions. A similar triangle for varying anion combinations was employed: NO_3 at top, PO_4 at lower left, and SO_4 at lower right.

The remaining two cultures lacking potassium develop larger plants without conspicuously brittle margins but with the light brown color in the basal portions of the plants.

The omission of magnesium (solutions 7, 22) affected the total area as well as the dry weights of the plants adversely, but in appearance these plants resembled adjoining cultures receiving a full complement of nutrient ions. The cultures lack-

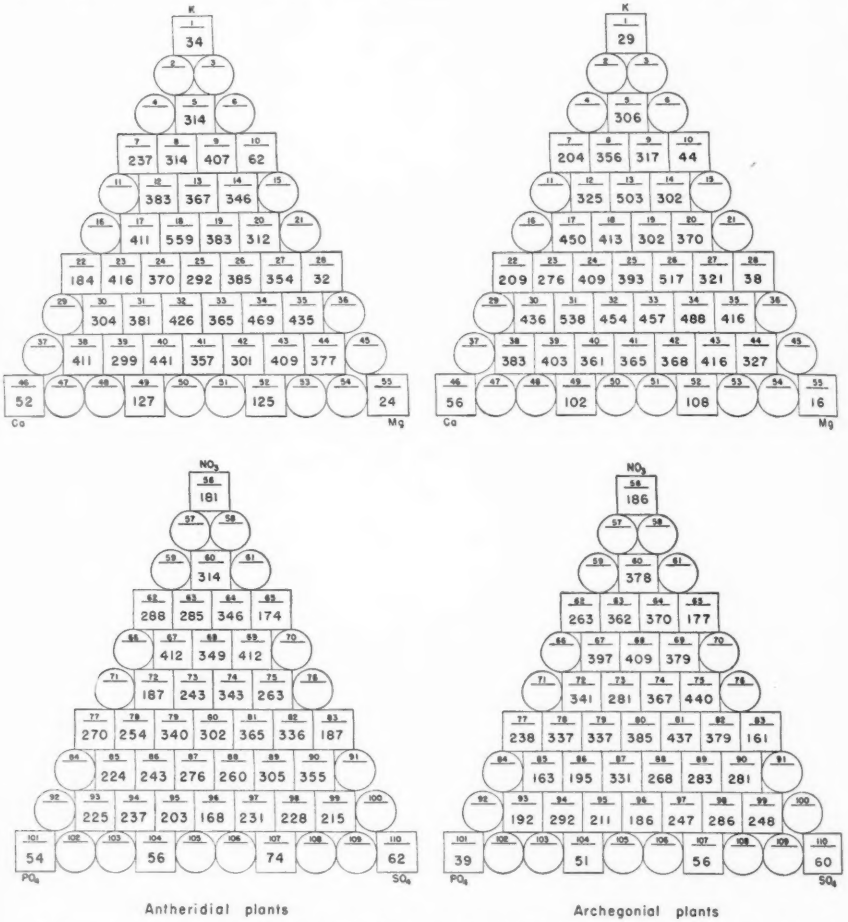

FIG. 2.—Area in square centimeters of six plants growing in each nutrient combination. Two triangles at left are male plants; at right, female.

ing magnesium and another cation responded according to the pattern usual for lack of calcium or potassium (solutions 1 and 46, respectively).

ANION TRIANGLE.—When planted on September 25, each cutting averaged 3 sq. cm. in area. Plants growing in solutions containing no nitrate (101, 104, 107), no phosphate (56, 65, 83), or both (110), developed darker midribs; and scales,

rhizoids, and lower epidermis became red after about 10 days. In all these positions algae were nearly absent from the surface of the glass cloths and of the solutions during the entire experiment. After about 2 weeks the plants lacking nitrates failed to gain in size, remained relatively narrow, produced practically no new gemmae cups, and forked dichotomously only infrequently, so that apical growing points were relatively few in number. The color of the upper surface of the plants became more yellowish as time went on.

The omission of phosphorus resulted in a different response after 2 weeks. Plants possessed a very dark midrib region, and the intensely green color of the upper surface was superimposed on the red color of the under surface. Dichotomy occurred frequently, giving the plants a rosette-like appearance. The plants were wider than average, and gemmae cups were very numerous (fig. 6). Another characteristic of plants lacking phosphorus was the tendency to be closely appressed to the glass cloth. Whether this is a phototropic or a geotropic response, or influenced by chemical or by mechanical stimuli, has not been determined. Only two antheridiophores formed on plants growing in the entire anion triangle—one each on solutions 65 and 69, the former lacking and the latter low in phosphorus.

Omission of sulphate from the solutions failed to alter the gross appearance of the plants and influenced dry weights only slightly (fig. 3). There may have been sufficient sulphur present in the atmosphere, since no special precautions were taken to exclude greenhouse air from the cultures.

Response to combinations of all ions

Cation triangle.—Plants in the twenty-eight positions in the center of the triangle all continued active growth during the entire period. As in previous experiments, the majority of cultures surrounding the exact center (solution 25 in this experiment) were larger in area, or heavier on the basis of dry weight, or both, when compared with the central position. This observation possibly has no great significance until additional data are available. Dry-weight accumulation by both sexes was greatest in position 34 and had a nutrient supply of $\frac{2}{9}$ K, $\frac{2}{9}$ Ca, and $\frac{5}{9}$ Mg solutions. Each triangle of fifty-five possible positions was treated as a unit in assigning rank. With only thirty-seven positions in use, the greatest dry weight accumulation is designated as rank 1 (position 34 in the cation triangles supporting male and female plants, respectively) and the least dry weight as rank 37 (position 28 in the cation triangle of male plants and position 55 in the cation triangle of female plants—both positions from which calcium is absent). The uppermost triangles in figure 3 show that within the central part of the cation triangle the distribution of rank is fairly diverse, indicating that K, Ca, and Mg may be present in rather wide ranges of concentrations and combinations and still be suitable for healthy growth of *Marchantia*.

ANION TRIANGLE.—Of the twenty-eight centrally located positions, plants in the row receiving $\frac{1}{9}$ PO_4 (solutions 60, 64, 69, 75, 82, 90, and 99) appeared most robust after the first 2 weeks, with the exception of plants on solution 99. Plants

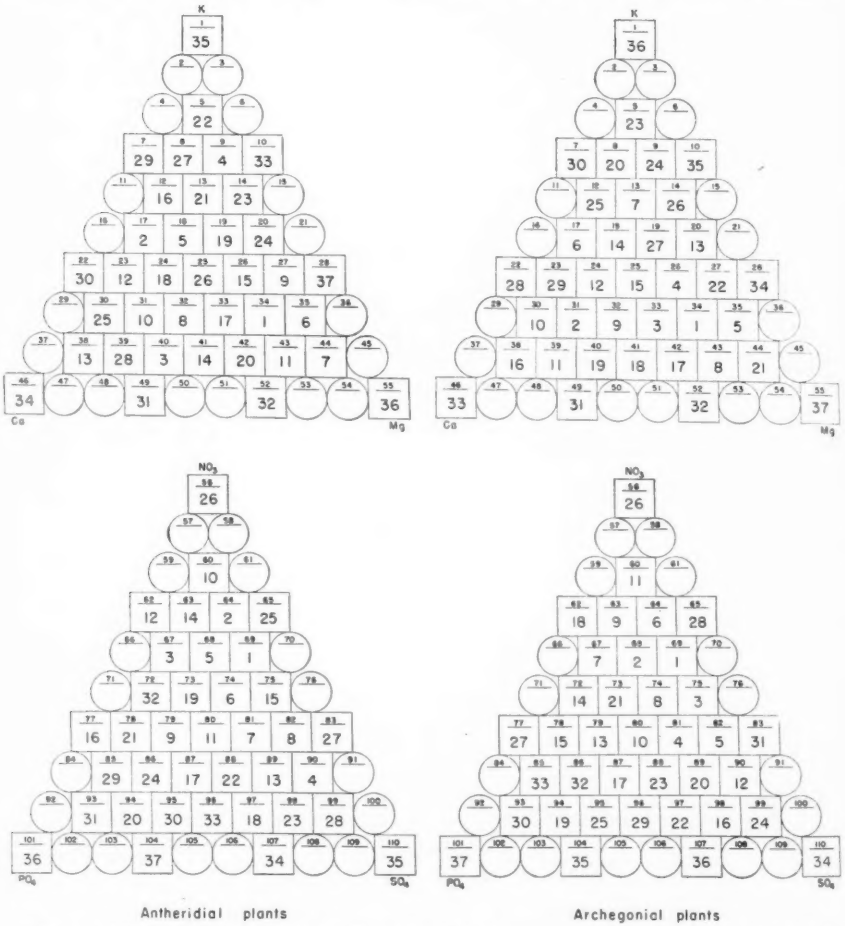

FIG. 3.—Rank on basis of dry weight. Two triangles at left represent male plants; at right, female. Each triangle is a unit for ranking. Rank 1, greatest dry weight, is in same position (no. 34) in cation triangle for male and also female plants. Similarly, rank 1 in anion triangles is in same position for both male and female plants (no. 69).

on solutions 64 and 69 made observably greater growth during the entire growing period, and on the basis of dry weight the latter ranked highest among male and female cultures in the anion triangle (fig. 3). Toward the base of the triangle,

where proportions of nitrates decreased progressively, plants were correspondingly smaller (fig. 2) and had the lowest final dry weights of the complete nutrient plants (fig. 3). Male as well as female plants accumulated the greatest dry weight in position 69, with a solution mixture of $\frac{5}{9}$ NO_3, $\frac{1}{9}$ PO_4, and $\frac{3}{9}$ SO_4. When all six ions were present and anions were varied, increased growth in area and greater accumulation of dry weight were definitely correlated with increased nitrate and decreased phosphate supply, even though the former was greatly increased and the latter decreased in the stock solutions of this experiment. These relationships are summarized in table 1 and figure 3.

Response to small amounts of calcium

After 24 days some of the plants in the row of the cation triangle receiving only 1 part of calcium to a total of 8 parts of potassium and magnesium began to develop black tips (fig. 4). Often the wings near the apical notch became black first, followed by darkening of the region of meristematic activity; or at times the region of the apical cell(s) suddenly became dark. A V-shaped zone soon developed progressively in a posterior direction, as shown in a plant tip near the upper center of figure 4, 20♂. On many plants the blackened areas became watery. By the end of the experiment, a few days after the first symptoms were noted, many of the blackened tips were dry, curled upward, and were brittle (fig. 4). No correlation seems to exist between this sudden death of the terminals and the factors which were obviously varied—the concentrations of potassium and magnesium. Since the only common factor in all these cultures was a $\frac{1}{9}$ Ca supply, it may be assumed that the low concentration of the calcium ion is responsible for these symptoms. Injury was observably greatest in male and female plants growing in solution 5. The following solutions are listed in the order of decreasing injury to the respective *Marchantia* cultures: 5♂ and 5♀ > 9♂ > 35♂ and 35♀ > 20♀ > 27♂ > 20♂ > 44♂ and 44♀. No external evidence of "burning" was noted in plants growing in solutions 27♀, 9♀, 14♂, and 14♀. In all the preceding cultures a $\frac{1}{9}$ proportion of calcium with reference to the other cations in solution is equivalent to a calcium concentration of 0.3 millimols per liter, or 12 p.p.m.

Anatomical effects of calcium deficiency

When calcium is absent or deficient in any particular plant, the anatomical effects on the cells and organs of that plant often are characteristic enough to be diagnostic for this element. Death of the stem apex (**3, 5, 6, 7, 8**), abnormalities and death of root tips (**1, 4, 7, 8, 12, 13**), hooking downward of the tip of young leaves in the bud (**5**), and yellowing of the youngest leaves (**7**) followed by necrosis of leaf tips and margins (**5, 6**) have all been reported for several flowering plants. Premature vacuolization and eventual loss of all cytoplasm in the elongating cells

FIG. 4.—Portions of archegonial plants (top) and antheridial plants (bottom) of *M. polymorpha* growing on nutrient combination 20, with Ca supply of $\frac{1}{8}$. Several plants of both sexes exhibit blackened tips, most of which turn upward. $\times\frac{3}{8}$.

of the root tips have been reported for Japanese millet, rape, buckwheat (**4**), wheat, corn (**1**), pea (**12, 13**), and other plants. In rape, cells of the cortex of the stem were large and the stele relatively small when compared with the cortex. In buckwheat the situation was reversed. A similar diversity of response was reported in the length of the palisade cells when calcium is withheld from these two plants (**4**). Roots of peas growing in the absence of calcium lack resistance to overcome infection by microörganisms (**13**).

When *Spirogyra* and *Zygnema* are grown on minus-calcium solution, the chloroplast loses its conspicuous lobing, becomes narrow, and soon lacks starch. Excessive vacuolization was followed by general cytoplasmic disorganization, although even after 6 weeks nuclear division was reportedly unaffected but the wall between newly formed nuclei failed to form. After 2 months of calcium absence the apical cell of a *Spirogyra* filament was the first to possess a disorganized chloroplast and to contain a dark substance related to the lecithins (**9**).

The responses of the gametophyte of *Marchantia* to deficiency or lack of calcium were investigated in detail anatomically. Thallus tips from plants receiving only $\frac{1}{9}$ Ca (solutions 5, 9, 14, 20, 27, 35, and 44), as well as tips from initial control plants growing on a complete nutrient stock solution, and a few tips from cultures surrounding the low-calcium triangle positions, were imbedded in paraffin, sectioned at 7 μ, and stained in Flemming's triple stain. Nearly all tips were sectioned longitudinally. Oblique and transverse sections of the tip confirmed observations made on longitudinal ones. Transverse sections of older parts of the plant are of less value in this phase of the study, since nearly all cells a few millimeters back of the growing tip are mature and practically devoid of cytoplasm and much resemble cells of healthy plants. The walls of the cells of the older portions often stain intensely with methyl violet when the cells of the tips of the same lobe are necrotic. DAY (**2**) found only slight variation between transverse sections of pea roots taken from healthy and calcium-deficient plants, but the amount of elongation was distinctive.

Plants growing as initial controls on a complete nutrient solution possess an apical growing region characterized by much meristematic activity. The apical cell is not clearly distinguishable from many of the surrounding cells (fig. 5, control ♀). The meristematic zone is several cells wide and merges gradually into the zone of cell enlargement and maturation. Cells and air chambers of the dorsal photosynthetic layer differentiated gradually from the surface layers of the apical meristem (fig. 5, control ♀). In the same treatment ($\frac{1}{9}$ Ca supply) not all plants responded alike; some were entirely necrotic whereas others showed slight injury. As a result, tips taken at random might not represent the state of an entire culture accurately. Many sections from cultures showing a deficiency of calcium possessed degenerating cells (fig. 5, 14♀, 9♂, 35♀). Longitudinal sections of black-

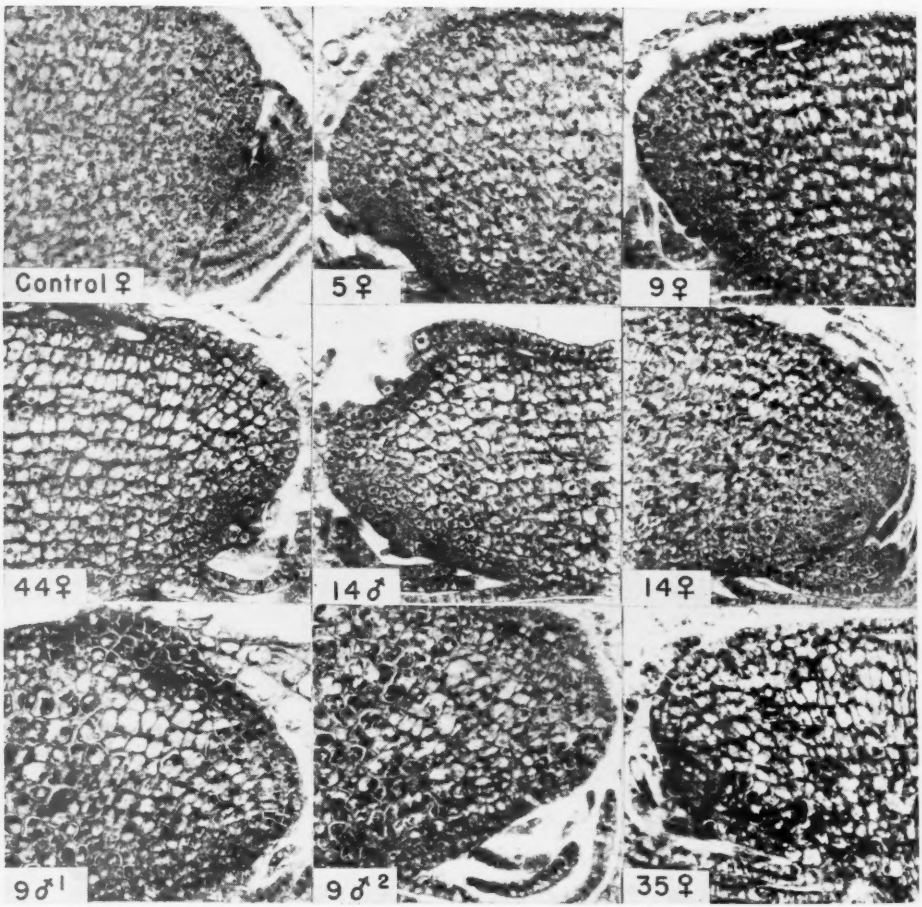

FIG. 5.—Vertical longitudinal sections through apical notch of *M. polymorpha*. Control is growing as stock plant on complete nutrient solution. Numbers indicate position in triangles. Apical cells show most clearly in 44 ♀, 14 ♂, 14 ♀, and 35 ♀.

ened tips showed only advanced stages of terminal degeneration, with no recognizable apical cell or even apical notch area. All sections shown in figure 5 were from tips which possessed no external evidence of necrosis, such as discoloration, and no collapse.

First evidences of unhealthy conditions found in longitudinal sections through the tip of a calcium-deficient plant are the larger vacuoles in the cells of the maturing region, the darker-staining nuclear zones, and the smaller number of chloroplasts. These conditions are shown in figure 5, position 9♀ (and to a lesser degree position 5♀), even though plants in the former position possessed no outward evidences of unhealthy development, while many plants in the latter culture had blackened tips. REED (**10**) describes the premature vacuolization and polarization of meristematic cells of the stem tips of apricot and peach trees and the accumulation of phenolic materials in maturing cells when zinc is deficient, but subsequent necrosis when this element is lacking or deficient is not reported.

Associated with the internal symptoms just noted, the meristematic zone is limited to a few concentric layers of cells derived from the apical cell, all containing a relatively large proportion of protoplasm and small vacuoles (fig. 5, 9♀ and 5♀). When such abrupt zonation becomes especially evident, the apical cell is easily distinguishable (fig. 5, 44♀ and 14♂). Meristematic activity continues in the dorsal region, where air chambers develop (fig. 5, 44♀). Gemmae-cup differentiation also continues (fig. 5, 14♂), even when cells below the single floor layer of the cup are mature—as shown by large cell size and extreme vacuolization. The ventral segment of the apical cell in position 44♀ (fig. 5) is in the process of transverse division, but the spindle is not clear because of the reduction in size of this figure.

Following the period of limited meristematic activity with concomitant preponderance of mature cells, necrosis in the dorsal areas (near the tip of the plant) occurs (fig. 5, 14♀). At times this zone of dead cells involves an embryonic gemmae cup (fig. 5, 9♂[1]). The breakdown of dorsal tissues is in contrast to the longevity and persistence of the small ventral cells which surround the bases of smooth rhizoids and are the source of regenerated thalli (**15**).

Most tips in which necrosis is beginning possess apical cells which apparently are still functioning. Sections of a single tip in position 9 (fig. 5, 9♂[1] and 9♂[2]) show that dichotomy is still in progress, since different apical cells are present in these sections, which are located on one microscope slide. One section apparently has a living apical cell, while a few microns farther on (fig. 5, 9♂[2]) the other half of the dichotomy possesses a necrotic apical cell region and a zone of living cells forming a V just posterior to the apex. As in position 35♀ (fig. 5), all cells of the tip eventually die.

The next event usually is collapse of the cells of the tip, accentuated or even

initiated by decay. Cells in the interior of the older portions of the thallus collapse (**15**, figs. 11, 12). Regeneration of plants from the small cells surrounding the base of smooth rhizoids located a few millimeters posterior to the tip has been described previously (**15**).

The rank of male and female plants in the upper two triangles of figure 3 indicates that, despite the unhealthy internal condition of most of the tips in the $\frac{1}{9}$ Ca row, accumulation of dry weight was better than average and in some instances nearly maximum for this experiment. Until the tips began to blacken, the gross appearance of these plants was among the best in the cation triangle.

Vegetative characters correlated with sex

In experiments 8 and 9 (**15**), where antheridial and archegonial plants were not grown simultaneously, the number of gemmae cups on the female plants was considerably less than on the male plants (568 on ♀, 2300 on ♂; a ratio of 1:4), but no significance was expressed since the data were insufficient to warrant a conclusion. By growing both sexes simultaneously under comparable conditions in the experiment under discussion, the earlier results were confirmed (946 gemmae cups on female, 6268 on male plants; a ratio of 1:6.6) and a rough correlation between sex and gemmae-cup production was established. Total gemmae-cup counts for each culture, sex, and triangle are given in figure 6.

Other factors being equal, decrease of the phosphate supply tends to favor greater gemmae-cup production. When all phosphates are absent, however, the number of cups on male plants tends to decrease while on female plants the number increases. Cup ratios are average when phosphates are present in as little as $\frac{1}{9}$ proportion (0.47 millimols per liter); but when phosphates are omitted the ratios are much lower, so that a male plant cannot be distinguished from a female on this basis. As shown in figure 6, decreased nitrate supply results in the production of fewer gemmae cups. Maximum number of cups seems to depend, therefore, largely upon a high nitrate level and a very low phosphate supply. When nitrate supply is high and all common ions are present in the nutrient solution, the sex of vigorously growing cuttings of *M. polymorpha* may be determined reasonably accurately by observing the thallus width approximately 1 cm. back of the apical notch and of the wings. Male plants are narrow when compared with female plants.

The upper surface of vigorously growing female plants is smoother and reflects light more readily than similarly cultured male plants. Whether this appearance is dependent upon differences in cuticle, size of air chambers, curvature of air-chamber roof, relative protrusion of chimney cells of the pore, or upon a combination of these factors is not yet determined.

In general the margins of the male plant are more undulating and tend to curve downward abruptly, especially if infested with blue-green algae or growing in a

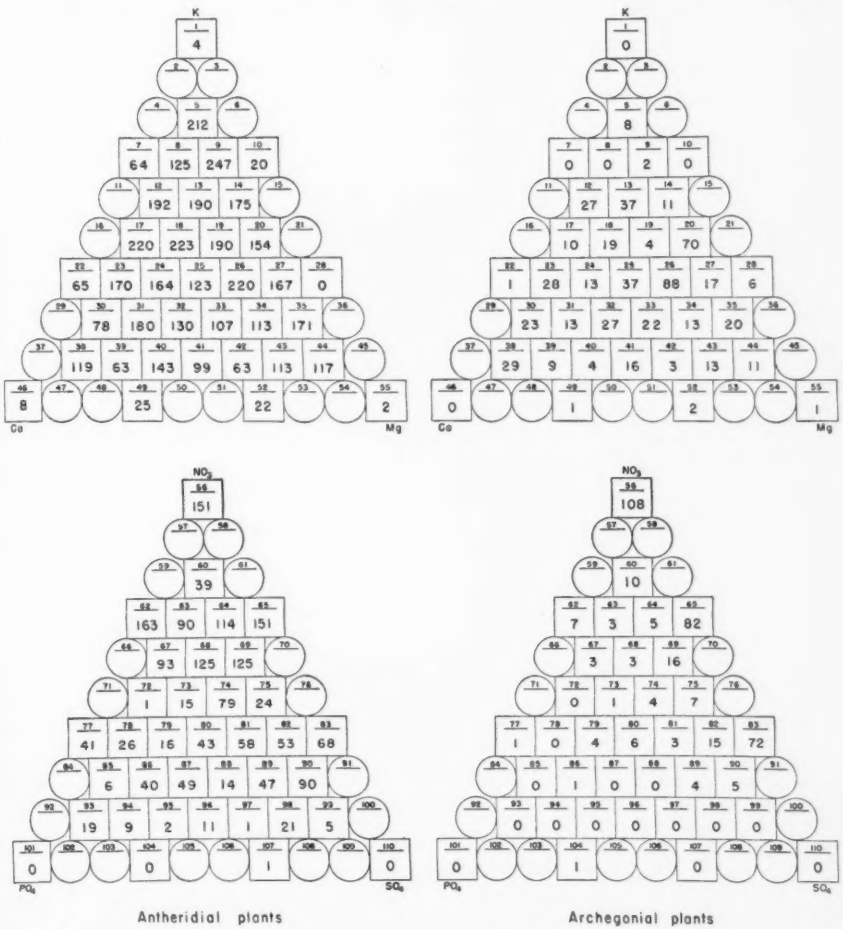

FIG. 6.—Number of gemmae cups on six plants of each culture. Counts on male plants in two triangles at left, female at right. Counts include cups present on original cutting. Ratio of cups on all male plants to those on female is 6.6:1. In anion triangle greatest cup number is associated with decreasing amounts of PO_4 in nutrient solution. Omission of PO_4 results in very slight differences in number of cups on male and female plants. Larger numbers of gemmae cups and higher nitrate supply correlated positively.

medium with a high pH. Often the marginal row of ventral scales tends to curve out and up, forming a wavy white margin in the male plants. Under these conditions, the smaller number of gemmae cups, the broader thallus tip, and the plane

surface of the female plants, known as culture B, serve to distinguish it from culture A, the male plants. These distinctions have definite taxonomic value in the clones just mentioned. Even with a low calcium supply (fig. 4), the distinction between culture A (bottom) and culture B (top) is possible on the basis of gemmae-cup number and gross appearance. Cultures of *M. polymorpha* plants from widely scattered localities in the United States are now being studied to determine the validity of these observations on other clones and to determine a possible correlation between sex and the relative number of gemmae cups produced by any particular culture. If a positive correlation is found, indicating possible sex linkage, further inquiry into the mechanisms of gemmae-cup initiation and factors influencing their development would be highly desirable. If maleness and the presence of large numbers of gemmae cups are not associated in other clones, an extensive field of genetic investigation would be opened. Similarity in number of gemmae cups on both male and female gametophytes when phosphates are absent indicates a need for study of the possible relation of this ion to the factors concerned in the expression of sexual characters.

Gametangiophore production

On the 888 plants grown during the present study, only eleven antheridiophores and three archegoniophores differentiated. This response is to be expected, since light intensity decreased during the term of the experiment (September–October).

Summary

1. As in previous experiments with *Marchantia polymorpha*, the omission of K, Ca, NO_3, or PO_4 ions results in characteristic differences in the gross appearances of the plants. The lack of K produces plants with tan-colored bases and slightly narrower tips. Absence of Ca results in almost immediate death of the growing tips. Deficiency of NO_3 and PO_4 is indicated by reddening of scales, of rhizoids, and of lower epidermis. Plants lacking the former ion become light green, possess few gemmae cups, and fork infrequently; but plants growing on solutions lacking PO_4 soon become dark green, have abundant gemmae cups, and because of frequent dichotomy are rosettes. Deficiencies of Mg and SO_4 are not indicated by any characteristic symptoms.

2. With a concentration of 0.3 millimols of calcium per liter of solution, death and degeneration of the plant apices result. Microscopically an early and rapid vacuolization occurs in enlarged cells near the apical cell. Necrosis first begins in the more nearly mature cells and continues until the entire tip is dead.

3. When grown on glass cloth and supplied with abundant nitrates, plants with many gemmae cups, narrower thalli, and incurved margins are male, belonging to culture A. Plants of culture B (female) have fewer cups, broader thalli, and plane surfaces.

4. On the basis of dry-weight accumulation and total area of plants, optimum vegetative growth is evident in certain areas of each triangle. On this basis, and with consideration of the gross and microscopic aspects of the plants, molar concentrations of the six ions may be suggested as meeting the requirements of *M. polymorpha:* K 0.0012, Ca 0.0007, Mg 0.0014, NO_3 0.0034, PO_4 0.0004, and SO_4 0.0008 mols per liter. In practice, a solution may be made up using the following quantities of a 0.5*M* solution of each of the following salts: KNO_3 1.6 cc., $Ca(NO_3)_2$ 1.4 cc., $Mg(NO_3)_2$ 1.2 cc., KH_2PO_4 0.8 cc., and $MgSO_4$ 1.6 cc. per liter of solution. Slight variations in the proportions of these salts do not affect the mineral requirements of this plant.

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EFFECT OF PHOTOPERIOD AND TEMPERATURE ON DEVELOPMENT OF BARLEY

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(WITH TWO FIGURES)

Introduction

The role of artificial light used to extend a natural photoperiod is different in short- and long-day plants in that it prevents flowering in the former and induces it in the latter. These opposite responses to the same stimulus suggest that the basic reactions influenced by supplemental light may be different in the two cases. One source of evidence for or against this assumption would be found in comparison of the intensities of light required to produce these characteristic responses in typical long- and short-day plants. Most of the data available for such comparisons, however, come from experiments dealing with the practical aspects of the use of supplemental light in the control of flowering. The records from most of these are based on the final presence or absence of open flowers or large buds in the experimental material and do not deal with the early stages of floral initiation. Such records are entirely satisfactory for the purpose for which they were intended, but they are not useful for the comparisons here proposed. They show that certain light treatments result in failure of the plants to flower but give no indication of the reason for the failure.

Early examination of the experimental material by dissection methods is required to determine whether the lack of flowers results from failure of flower buds to be initiated or failure of flower primordia to continue development after they have been initiated. For short-day plants, particularly Biloxi soybean (**2, 6**), such data are available, but this is not the case for long-day plants. The ultimate collection of such data from various typical long-day plants was one of the objectives of undertaking detailed studies with barley.

In addition to being generally regarded as long-day in their responses to photoperiod, several cereals, including barley, are reported to exhibit clear-cut interactions between temperature and photoperiod. In these cereals the effects of temperature treatments applied during germination or seedling stages may express themselves during the subsequent development of the plants or at their maturity. Since the cereals may exhibit after-effects of temperature treatment as well as responses to photoperiod, they are well adapted for varied experiments involving either of these factors or their interactions.

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The purpose of these experiments was to study the influence of different photoperiods upon the development of barley seedlings previously subjected to various temperature treatments. The results presented in this paper were obtained from experiments conducted in the springs and summers of 1940 and 1941.

Literature review

GARNER and ALLARD have reported that long photoperiods hastened the heading of wheat, oats, barley, and rye (3). In the winter cereals, however, PURVIS and GREGORY (7) have stated that flowering was accelerated by an initial treatment with either short photoperiods or low temperature, but they emphasized the fact that, in either case, final treatment with long photoperiods was necessary for the completion of flowering. In spring rye, on the other hand, they found that short photoperiods resulted in the formation of increased numbers of leaves before heads were formed, and that heading was thereby delayed by such treatment. It seems desirable, therefore, to regard these cereals as "long-day" plants, as PURVIS and GREGORY suggest, since the heading of both spring and winter varieties is accelerated by the application of long photoperiods, except during the very early stages of the winter ones, when short photoperiods seem more effective.

Both PURVIS and GREGORY (7) and MCKINNEY and SANDO (5), working with rye and wheat, respectively, have reported that the number of leaves formed before spikelet primordia were differentiated was influenced by the temperature during germination and the photoperiod immediately following. Long photoperiod was correlated with lower numbers of leaves and short photoperiod with higher numbers. In spring rye, however, PURVIS and GREGORY found that leaf number was not influenced by germination temperature, while MCKINNEY and SANDO, working with spring wheat, obtained opposite results. The response of spring barley to this factor was not discussed by either group of workers.

The development of the barley spike has been well described and illustrated by LERMER and HOLZNER (4) and by BONNETT (1). BONNETT reported that four true leaves were differentiated in the ungerminated seed, the fourth and youngest consisting of a transverse ridge near the growing point of the embryo. BONNETT's plants developed a total of approximately twelve true leaves from the main axis, before the primordia of spikelets were initiated. This was a somewhat higher number than was produced by the plants in the present experiments, the difference undoubtedly being related to the different environmental conditions of the two experiments.

Plan of experiment

Two varieties of barley were selected, one a strictly winter type and the other a semi-winter type. The former, Kentucky no. 1, C.I. no. 6050, is reported not to head unless it receives a period of low temperature during its early development,

while the latter, Wintex C.I. no. 6127, is known to require little or no low temperature. In these experiments the Kentucky plants failed to head in any of the experimental lots but the Wintex plants headed under all treatments. This report, therefore, deals exclusively with the results obtained from Wintex.

All the plants were started in rooms where they were grown for various numbers of days under constant conditions of light intensity, temperature, and photoperiod. While the plants were in the rooms they were all grown at 65° F. and on a daily photoperiod of 16 hours. The light source was a carbon arc supplemented with about 160 foot-candles of Mazda light. The total intensity at the surface of the leaves was approximately 2000 foot-candles. Beginning May 7, 1940, plantings of both varieties were made at four successive 5-day intervals. Thus on the twentieth day after the first planting, seedlings 5, 10, 15, and 20 days old were available. Differential temperature treatments were started at this time. Three temperatures, 35°, 45°, and 65° F., were provided in separate control rooms. The seedlings of each planting date were divided into three equal lots, and one lot was placed in each room.

While the plants were receiving differential temperature treatment the photoperiod was continued at 16 hours daily, as it had been during their earlier growth, and the quality, duration, and intensity of the light were the same over all lots. Although an attempt was made to hold the temperature of the coldest room at 35° F. continuously, this was not possible during all of each photoperiod because of considerable heat liberated by the lights. During the dark periods the temperature was held at about 35° ± 2° F. and during the photoperiods it was always less than 45° F. Temperatures of the other two rooms did not deviate more than ± 2° from 45° and 65° F., respectively.

The temperature treatments were continued one week; then the plants were transferred to an outdoor area where facilities were available for control of photoperiod. The plants of each treatment were divided into four groups, and those of each group were transplanted in duplicate lots of five to separate photoperiods. The plants were placed in boxes of soil $3\frac{1}{2}$ feet long, 1 foot wide, and 1 foot deep, with two rows of five plants in each box. The boxes were mounted on trucks to permit movement into and out of darkhouses to control length of photoperiod. At the time of transplanting, the various lots were 12, 17, 22, and 27 days old.

Photoperiods of 12, 16, 20 hours, and continuous light were employed. All trucks were removed from the houses at 6:00 A.M. daily and returned at 6:00 P.M. Those on 12-hour photoperiod were placed in darkness at 6:00 P.M. but all others were subjected to Mazda light inside their respective houses until the desired length of photoperiod had been attained. In the 16-hour chamber the lights went out at 10:00 P.M.; in the 20-hour chamber they went out at 2:00 A.M.; while in the continuous-light room the lights were on from 6:00 P.M. to 6:00 A.M. All lots

thus received the same amount of natural light each day but differed in the amount of artificial light received. Since the intensity of artificial light, 30–40 foot-candles, was very low in comparison with that of natural light, the total amounts of radiant energy received by plants on long and short photoperiods did not differ greatly.

Following transplanting, daily records were made of the number of days from planting to awn appearance on the individual plants. These data were recorded when the first visible awn emerged from the boot. In the statistical analysis of the data these records were grouped into totals per row. The results are reported in the tables as means per plant.

The plants remained under the differential photoperiods until the grain on the 16-, 20-, and 24-hour photoperiods was mature. At this time all the plants were harvested, even though those on 12-hour photoperiod were still green and making vegetative growth. The roots of the plants were discarded and the entire tops dried at 80° C. The dry weight of the tops per lot of five plants was first determined. The heads were then threshed and the yield of seed determined.

The treatments in each of the four dark chambers available in the outside area were arranged in duplicate series and randomized within groups. In the analyses of the results these two groups were treated as duplicates, although strictly speaking the photoperiodic conditions were not duplicated. To have duplicated each condition would have necessitated a reduction in the number of kinds of photoperiodic treatments that could have been conducted. It was felt more desirable in this first experiment to retain the four photoperiodic conditions and to assume that differences between dark chambers were slight rather than to provide a measure of this factor through duplication of half as many kinds of treatments. The results were subjected to variance analysis and, in the discussion, where significance is attached to any differences reported, the odds were 99 to 1 or higher.

The variables studied in the experiment included four planting dates, three temperature treatments of 1-week duration during the seedling stage, four conditions of photoperiod during the later development of the plants, and duplicate lots throughout. This represents 48 kinds of duplicated treatments, or 96 separate lots. Each lot contained five plants, so the entire experiment required 480 plants of each variety.

So many plants were required that space in the control rooms was not available to produce extra plants for dissection during their early stages prior to and following the temperature treatments. To determine the approximate condition of the seedlings at various ages, another planting was made the following year and used exclusively for that purpose and for photographic records. The conditions of light intensity, temperature, and photoperiod in the rooms were made identical with those in the first experiment. This second experiment was planted on May 6, 11, 16, and 21, 1941, one day earlier than the 1940 experiment. The same time of

year was selected so that plants not needed for dissection could be transferred outside under conditions comparable with those of the previous year. The plants grown outside in 1941, however, were transplanted to 6-inch pots instead of to boxes of soil, to permit regrouping for photographic purposes. All photographs shown are from the 1941 experiment.

After the plants were dissected they were preserved in a mixture of formalin-alcohol-acetic acid until they could be photographed. Before photographing they were removed from the preservative, washed, and then placed in an aqueous solution of methyl green. As soon as the primordia had acquired a deep blue color they were ready for photographing. The material did not overstain objectionably if it was left in the stain for several days.

The stained material was photographed under about half an inch of water, where it was held in position by a needle inserted in the base of the stem. The needle was mounted in a universal joint in such a way that the material could be readily rotated in any plane. The whole assembly could be raised and lowered with a rack and pinion. Most of the photographs were made at a magnification of 12 diameters with a 32-mm. micro-Tessar lens mounted in a 5 × 7 camera. The primordia were illuminated by two small beams of light directed obliquely downward at them in such a way that excess light did not illuminate the black background at the bottom of the tank. This method of handling the material makes possible the collection of many samples in a short time, the photographic records being completed as time permits.

The data include dissection records made at the beginning and end of the temperature treatments, mean number of days from planting to awn emergence, dry weights of mature plants and of seed per plant, and mean number of seeds per plant.

Results

Under the conditions of these experiments the seedlings of all lots emerged from the soil 4 days after planting. The stage of development attained by representative plants of each lot when 5, 10, 15, and 20 days old is illustrated in the first column of figure 1. Beginning at this time the plants were subjected to three different temperatures for one week. At the expiration of the week representative plants were again photographed. Those of each planting that had received 7 days at 65° F. are shown in the second column of figure 1; the ones that received 45° F., in the third column; and those that received 35° F. are shown in the last column. Corresponding lots of plants held at the two lowest temperatures did not differ greatly at the end of a week of treatment. Plants of both lots, however, were definitely smaller than those of the lots that had been continued at 65° F. during this time.

At the time the temperature treatments were started the oldest seedlings had formed only five visible leaves. The formation of new structures at the growing points of the main axes of these seedlings, however, was already complete. The average number of nodes in the main axes of the eight plants dissected was 32.5 (table 1), and in the plants dissected a week later there were no additional structures present.

Plants of the three later plantings had not yet completed the differentiation of new structures in their main axes when the temperature treatments were started. During the week of temperature treatments all lots continued differentiation, but the rate was much greater in the older than in the younger seedlings. By the time the temperature treatments were completed all the plants of the second planting had practically completed the differentiation of new structures in their main axes.

TABLE 1

EFFECT OF LOW TEMPERATURE ON PRODUCTION OF NODES IN WINTEX BARLEY SEEDLINGS OF VARIOUS AGES. AVERAGES BASED ON 8 PLANTS

Age (in days) at beginning of temperature treatment	Total nodes at beginning of temperature treatment	Total nodes after 7 days' treatment		
		65° F.	45° F.	35° F.
20	32.5	31.4	32.1	32.4
15	23.1	32.3	31.8	30.6
10	9.1	31.9	15.3	12.6
5	5.0	14.7	7.4	6.9

The plants of the 65° lot of the third planting were also nearly complete at this time, but the two low-temperature lots of this planting and all lots of the last planting had fifteen or fewer nodes per plant when transplanted.

The beginning of spikelet formation in plants grown continuously at 65° F. occurred between the tenth and twelfth days (fig. 1). On the tenth day the collar-like rudimentary leaves found at the base of the spike were just forming. On the twelfth day spikelet primordia were conspicuous at six or more nodes of the rachis above these leaves. The rate of development of the young spike proceeded rapidly under the conditions of these experiments. Stages of development of representative plants grown continuously at 65° and of the growing points dissected from these are shown in the first two columns of figure 1. In the first column the plants with their growing points were 5, 10, 15, and 20 days old from top to bottom, and in the second column they were 12, 17, 22, and 27 days old.

The most obvious effect of the two low-temperature treatments was to delay development of the spikes. In the younger plantings the low temperature delayed

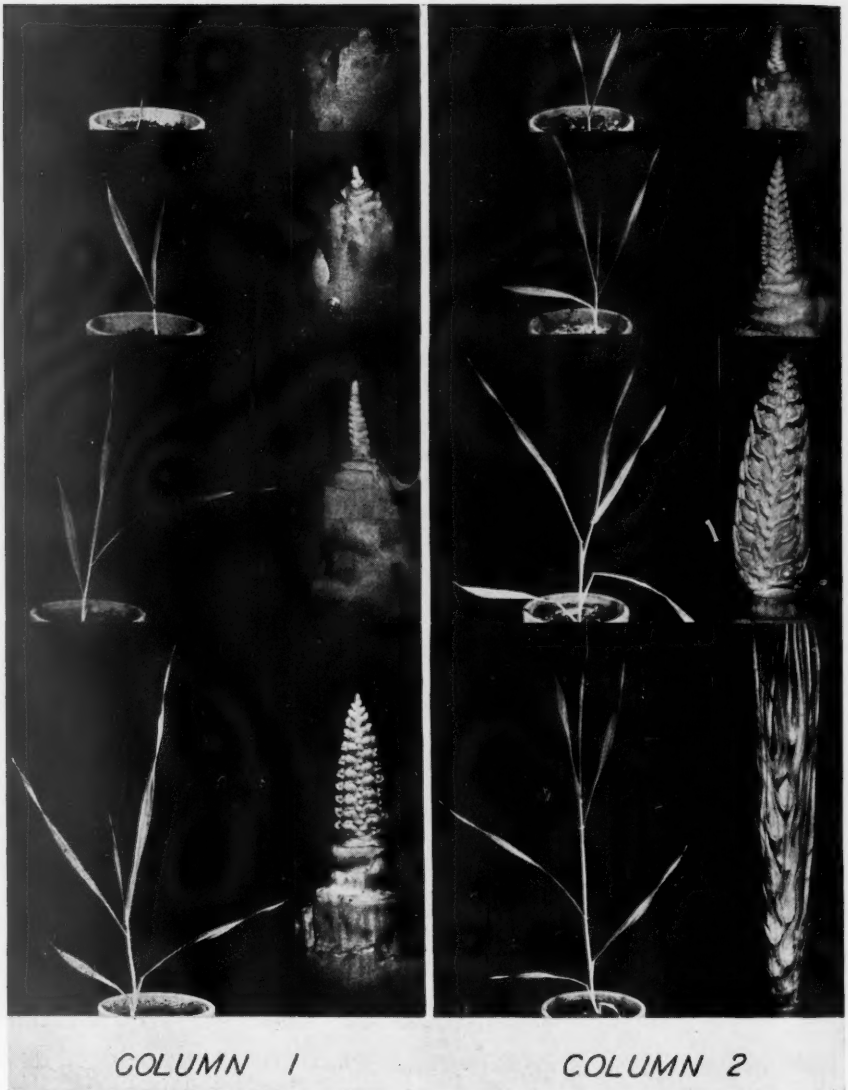

FIG. 1.—Seedlings from four successive plantings of Wintex barley grown on 16-hour photoperiods and under several conditions of temperature; last planting in top row and first in bottom row. Enlargement of terminal of main axis shown at right of seedling from which it was dissected. Seedlings of first column 5, 10, 15, and 20 days old, respectively, from top to bottom. Seedlings of second, third, and fourth columns all one week older than corresponding seedlings of first column. All seedlings grown at 65° F. at all times except those of columns three and four, which were transferred to 45° and 35° F., respectively, one week immediately prior to photographing. Approximate magnifications: all seedlings $\times\frac{1}{6}$; enlarged head in bottom row of column two $\times 4\frac{1}{2}$; all other terminals $\times 9\frac{1}{2}$.

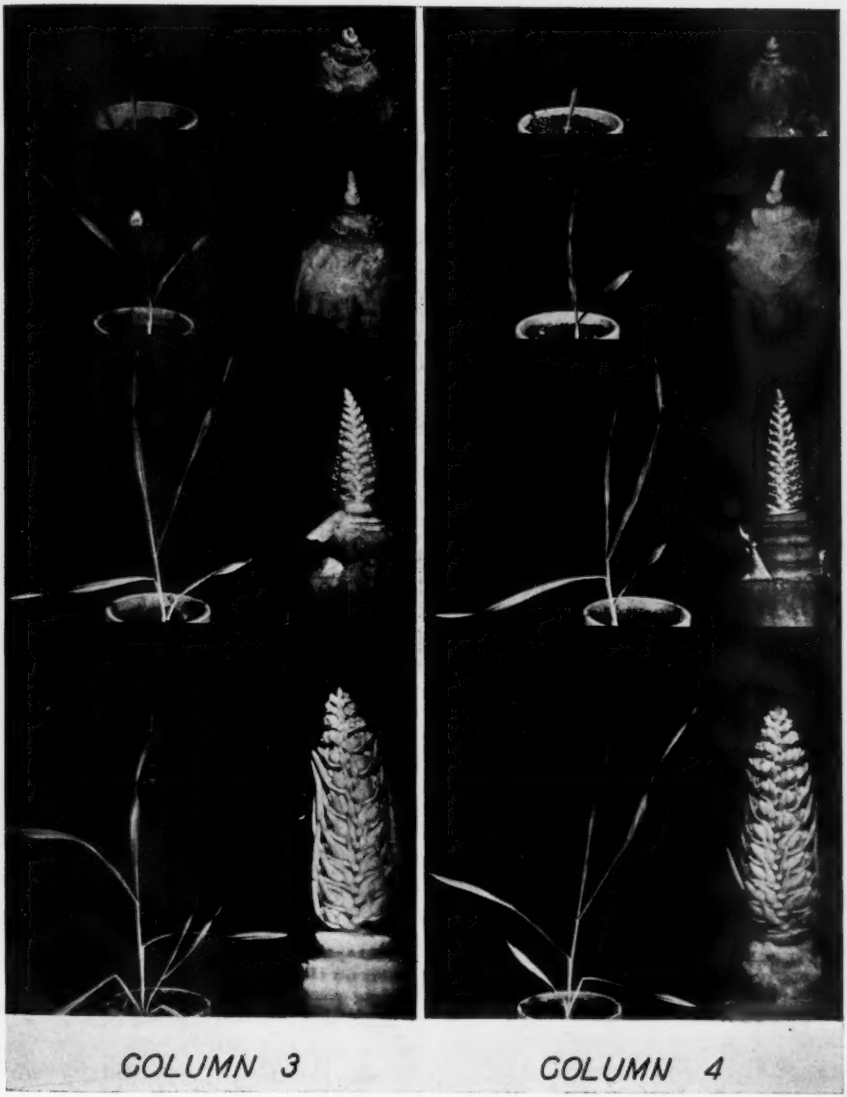

FIG. 1.—*Continued*

development of structures already present and retarded the differentiation of new ones. In the oldest planting the effect was only upon the rate of growth of primordia already established, because differentiation of new organs was complete before the low temperatures were applied. The amount of delay appeared approximately the same under both low temperature conditions (fig. 1, columns 3 and 4), but dissection data (table 1) indicate that it was actually somewhat greater in the 35° than in the 45° F. lot.

After transplanting to the boxes in the outside area, daily records were made on the appearance of awns on the main shoots of the individual plants, and photographs were made at frequent intervals. The stage of development attained by representative plants of the various lots on June 24, 22 days after transplanting, is shown in figure 2. At this time awns were visible on the plants of all treatments of the first planting and in some the heads had fully emerged from the leaf sheath. In the second planting awns were visible in all lots except the two low-temperature ones growing on 12-hour photoperiods. In the third planting no awns were visible in any of the 35° or 45° lots, but they had emerged in the 16-, 20-, and 24-hour lots of the 65° F. group. In the fourth planting at this time awns were visible only on the plants grown at 65° on 20- and 24-hour photoperiods.

The mean number of days required from planting to awn emergence varied with the photoperiod, age of plants, and temperature treatment (table 2). Of these factors, photoperiod produced the greatest effects. Awns appeared first on the plants receiving 24-hour photoperiod, and these were closely followed by those receiving 20- and 16-hour photoperiods. Awn appearance was delayed most in the plants receiving 12-hour photoperiods, particularly in those of the last two plantings. The plants from these two plantings, with the exception of the 65° F. lot of the third planting, had not completed differentiation of all spikelet primordia at the time of transplanting. The influence of short photoperiod on these plants could therefore affect both their further differentiation and their subsequent development and growth. Since differentiation of spikelets was complete in the plants of the first and second plantings and in the 65° F. lot of the third planting, short photoperiod affected the time of awn appearance only through its effect upon the growth and elongation of these previously differentiated structures. The character of growth of the plants receiving 12-hour photoperiods differed from that of the plants receiving 16-, 20-, or 24-hour photoperiods in that none of the elongating shoots ever grew completely erect and most of them were extremely prostrate. These plants formed many more tillers than those on the longer photoperiods, and although they produced spike primordia most of these tillers failed to elongate.

Awns emerged later in all plants that had received a week of low temperature during the seedling stage than in plants of similar lots grown continuously at

65° F. The effects of low temperature treatments on the time of awn appearance were correlated with the influence of these treatments in retarding the differentiation and development of the spikelet primordia previous to transplanting (fig. 1). When seedlings were transplanted to various long photoperiods these early-induced differences did not result in great variations in the date of awn emergence, but when they were transplanted to 12-hour photoperiod the date of awn appearance was delayed. This delay was greater the later the planting.

TABLE 2

EFFECT OF VARIOUS ENVIRONMENTAL FACTORS ON MEAN NUMBER OF DAYS FROM PLANTING TO APPEARANCE OF AWNS IN WINTEX BARLEY. AVERAGES BASED ON 10 PLANTS

Age (in days) of plants when differential temperatures were applied (grown at 65° F. on 16-hour photoperiod)	Temperature applied for 7 days just prior to transplanting (° F.)	Age of plants when transplanted to differential photoperiods and natural temperatures	Days from planting to awn emergence under differential photoperiods			
			12-hour	16-hour	20-hour	24-hour
20	65	27	35.1	35.2	35.1	35.0
15	65	22	36.7	32.8	31.8	31.9
10	65	17	41.6	31.8	31.0	31.0
5	65	12	46.8	32.0	30.6	30.2
20	45	27	39.2	36.6	36.1	35.5
15	45	22	47.1	37.6	36.2	36.1
10	45	17	57.7	36.9	35.5	35.2
5	45	12	64.9	36.3	33.5	32.4
20	35	27	39.9	36.4	36.4	36.2
15	35	22	45.5	36.8	36.0	36.0
10	35	17	56.5	38.0	35.7	35.8
5	35	12	78.7	37.0	33.7	33.4

The actual dates of awn appearance naturally varied from one planting to another. When the time of awn appearance was calculated from the time of planting, however, these differences were not great for any except the 12-hour photoperiod. The younger the plant when treatments were applied, the greater the retardation resulting from 12-hour photoperiod.

The mean dry weights of the plants were correlated with the photoperiods to which they were subjected after transplanting. The plants grown on 12-hour photoperiod were much heavier than those of any of the other lots (table 3). These plants produced no grain, in contrast to those grown on 16-, 20-, and 24-hour photoperiods. Their additional weight was due to the greatly increased leaf area resulting from the continued production of tillers throughout the entire growing season. Of the groups that produced grain, the 16-hour photoperiod was the most favorable for production of dry weight and the 24-hour photoperiod the least fa-

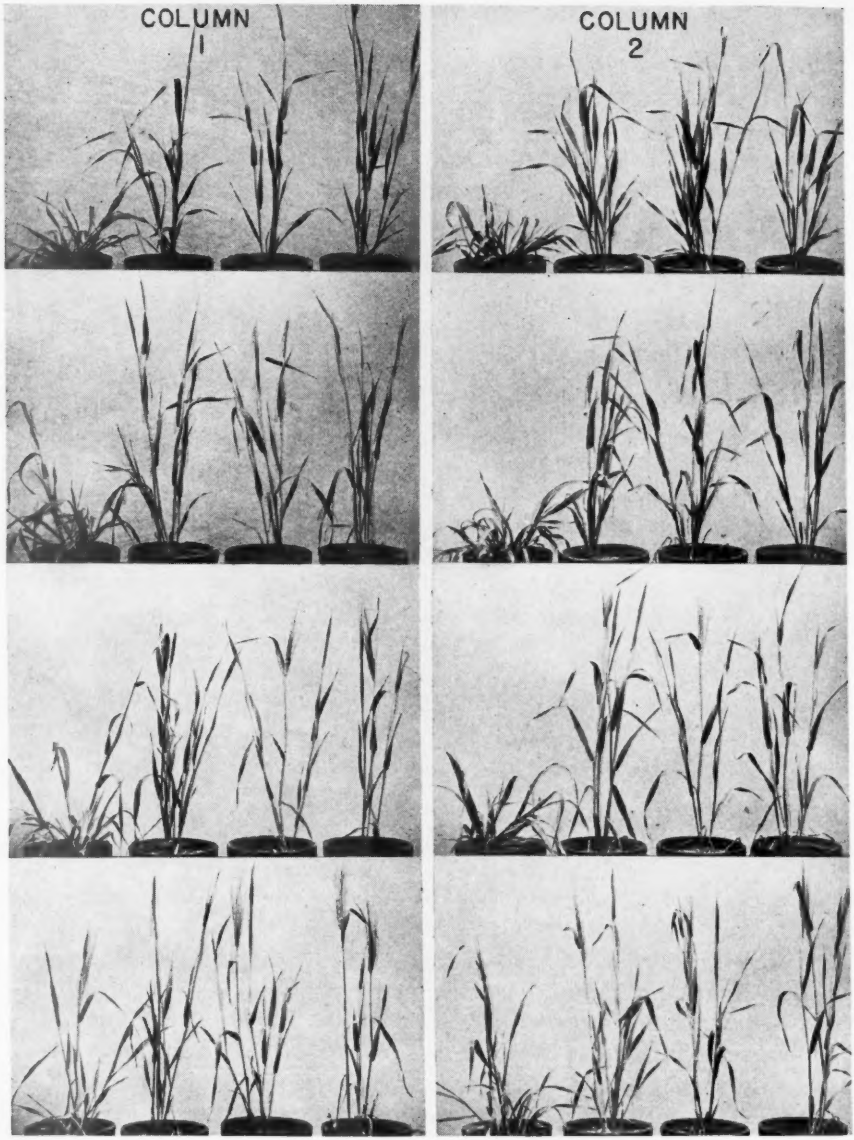

FIG. 2.—Wintex barley plants grown under three conditions of temperature and 16-hour photoperiod before transplanting, then shifted outside to prevailing temperatures and four different photoperiods. Plants from left to right in each group of four plants received 12-, 16-, 20-, and 24-hour photoperiods, respectively, after transplanting. Top row planted May 21, second row May 16, third row May 11, and fourth row May 6, 1941. All transplanted June 2. During week preceding transplanting, plants of first column received temperature of 65° F., those of second column 45° F., and those of third column 35° F. At all other times prior to transplanting all seedlings received 65° F. Photographed June 24, 1941.

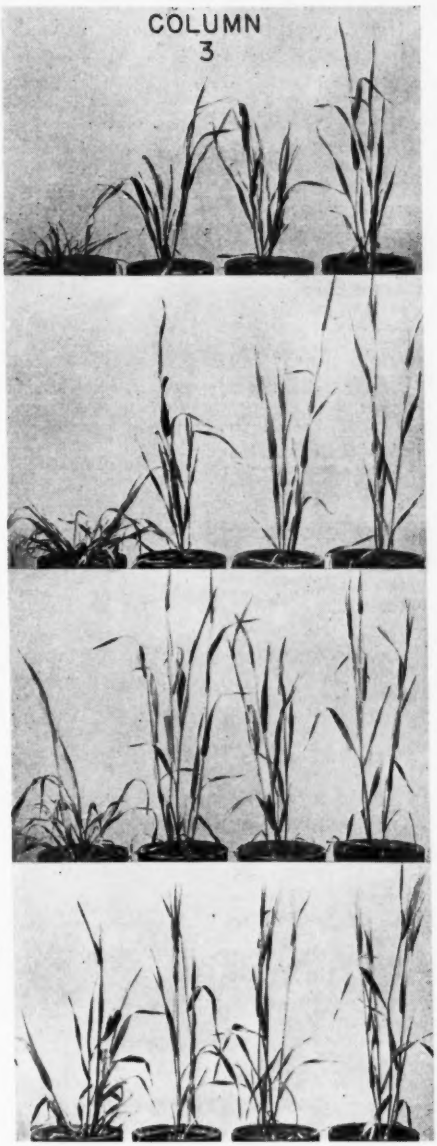

FIG. 2.—*Continued*

vorable. The differential temperature treatments applied before transplanting caused no significant differences in the accumulation of dry weight. The age of plants was not a factor in this respect since the plants were not harvested until the grain was mature.

TABLE 3

EFFECT OF VARIOUS ENVIRONMENTAL FACTORS ON MEAN DRY WEIGHT PER PLANT IN WINTEX BARLEY. AVERAGES BASED ON 10 PLANTS

AGE (IN DAYS) OF PLANTS WHEN DIFFERENTIAL TEMPERATURES WERE APPLIED (GROWN AT 65° F. ON 16-HOUR PHOTOPERIOD)	TEMPERATURE APPLIED FOR 7 DAYS JUST PRIOR TO TRANSPLANTING (° F.)	MEAN DRY WEIGHT IN GRAMS PER PLANT OF LOTS UNDER DIFFERENTIAL PHOTOPERIODS			
		12-HOUR	16-HOUR	20-HOUR	24-HOUR
20	65	9.4	3.7	4.1	3.4
15	65	10.2	6.3	4.1	4.0
10	65	12.4	6.1	6.8	5.7
5	65	18.2	6.0	5.5	4.5
20	45	11.2	3.7	4.7	4.6
15	45	12.8	6.3	5.8	5.1
10	45	15.2	6.5	5.6	4.8
5	45	13.9	6.3	5.9	4.1
20	35	9.7	4.1	4.5	3.5
15	35	10.5	5.0	4.0	3.4
10	35	11.9	5.2	4.3	3.5
5	35	12.5	5.6	4.3	3.5

TABLE 4

EFFECT OF PHOTOPERIOD WHEN APPLIED TO PLANTS OF VARIOUS AGES ON MEAN NUMBER OF SEEDS PER PLANT OF WINTEX BARLEY. AVERAGES BASED ON 10 PLANTS

PHOTOPERIOD	MEAN NO. OF SEEDS PER PLANT FROM LOTS TRANSPLANTED TO DIFFERENTIAL PHOTOPERIODS			
	12 DAYS	17 DAYS	22 DAYS	27 DAYS
16-hour	71	60	58	36
20-hour	50	52	41	41
24-hour	31	38	29	33

The number of seeds per plant was influenced by the length of photoperiod received after transplanting and by the age at time of transplanting, but not by the temperature treatment received prior to transplanting (table 4). The plants receiving 16-hour photoperiods produced the most grain while those receiving 24-

hour photoperiods produced the least; and the younger the plant at the time of transplanting the greater the yield. This fact was also true for the weight of grain per plant. Since these data show the same trends as those for number of seeds per plant, they are not presented.

Discussion

In barley and other cereals, differentiation of many of the structures found in the mature plant occurs during the first few weeks of seedling development. The rate of differentiation of these structures and the kind of structures found are both influenced by various environmental factors, including photoperiod and temperature. Even slight variations in length of photoperiod or germination temperature may result in the formation of different numbers of leaves before the initiation of spikes occurs. Differences in temperature also result in different rates of development. For these reasons different lots of plants that are not grown under controlled conditions seldom attain the same development at any given age.

In these experiments the conditions of growth prior to the time of transplanting were controlled and therefore reproducible at any time. The plants made vigorous growth and formed primordia of spikelets early in their development. The seedlings were all grown on a 16-hour photoperiod, and those held continuously at 65° F. completed the differentiation of all the nodes of the main axis 17 days after planting. The first primordia of spikelets were formed at the seventh or eighth nodes in nearly all plants. Since three or four of these nodes were present in the ungerminated seed, and one or two additional ones were formed before the seedlings emerged from the soil, it is evident that conditions favoring early spike formation approached the optimum.

Wintex barley resembles spring types in that it is capable of heading without the application of temperatures lower than 65° F. at any time during its development. The effects of the low temperatures applied to Wintex barley in these experiments were manifested through retarded growth rates rather than through after-effects of temperature typical of winter varieties. This retardation in growth as seen in the spike primordia (fig. 1) was very evident before the plants were transplanted to different photoperiods. Under each of the three lengths of photoperiod favorable to early heading these differences induced by low temperature were much less conspicuous by the time of awn emergence (table 2) than they appeared to be at the time of transplanting (fig. 1). Under a 12-hour photoperiod, which was less favorable to early heading, the retardations visible in the seedlings at the time of transplanting became still greater during the later development of the plants.

The awns of plants receiving 12-hour photoperiods appeared later than those of plants subjected to the other three photoperiods. This difference was slight in

the first planting but progressively greater in the later ones. This is to be expected, since the 12-hour photoperiod was applied to the late-planted seedlings earlier in their development, at a time when it could affect their differentiation as well as their growth. It is in this way that the chief influence of low temperature was expressed: it retarded the development of the seedlings so that when they were transferred to short photoperiods they were in an earlier stage of differentiation than others of the same age that had received no low temperature. Short photoperiod was therefore applied to them when they were in still earlier stages of differentiation; and its retarding influence upon awn emergence, acting upon the plants through a longer period of their development, served further to increase the original difference caused by the temperature treatment.

The differences resulting from photoperiodic treatments applied in these experiments are to be attributed to the influence upon the plants of supplemental light of relatively low intensity. Each photoperiod, regardless of length, contained 12 hours of natural light per day, so the main source of radiant energy was identical in all lots. The differences in length of photoperiod were the result of adding supplemental Mazda light of less than 40 foot-candles. Even when this supplemental light was used 12 hours per day, the total energy supplied was exceedingly small in contrast to that supplied by the 12 hours of natural light each day.

The greatest differences resulting from photoperiodic treatment occurred between the 12- and 16-hour lots. The 16-hour ones headed rather uniformly early in all plantings. The 12-hour lots headed nearly as early in the first planting but required progressively more time in each successively later planting. Although awns eventually appeared on every 12-hour plant in the experiment, none of these plants produced a single fertile seed. On the other hand, the plants of the 16-hour lots were all fertile and the heaviest yields of grain came from this photoperiod. These differences resulted from the presence or absence of approximately 40 foot-candles of Mazda light during 4 hours per day.

From these results it seems probable that still lower intensities might have been equally effective in promoting heading and subsequent maturation of seed. More detailed experiments concerning this point are under way.

Summary

1. Four lots of barley seedlings were grown on 16-hour photoperiod to ages of 5, 10, 15, and 20 days, respectively, at 65° F. Each lot was then divided into three groups and all were grown for another week under the same conditions of light and photoperiod. One group of each lot, however, was continued during the week at 65° F., one was transferred to 45° F., and the third to 35° F. The plants were then removed from the control rooms and transplanted out of doors for final differential photoperiodic treatment.

2. At the beginning and end of the temperature treatments representative plants from each lot were photographed and dissected. The total number of nodes in the main axis of each was determined, and enlarged photographs of the terminals of the main axes were made.

3. Ten plants from each age and temperature group were subjected to 12-, 16-, 20-, and 24-hour photoperiods after they were moved outside and remained on these photoperiods at natural temperatures for the duration of the experiment.

4. Number of days from planting to awn emergence was determined. At maturity the dry weights of the tops and of the grain were also determined, and counts were made of the number of seeds per plant.

5. Delay in awn emergence resulted from 12-hour photoperiod; the younger the seedling at the time of transfer to this photoperiod the greater the delay. Delay in awn emergence resulted from low temperature applied during the seedling stage. The greatest delay occurred when the youngest seedlings were subjected to a week of low temperature and were subsequently grown on 12-hour photoperiods. Typical temperature after-effects were not observed; the influence of low temperature was expressed through its effect on the growth rate while it was being applied.

6. The plants receiving 12-hour photoperiod did not produce a single fertile seed, but they produced more dry weight than the lots receiving longer photoperiods. The 16-, 20-, and 24-hour photoperiods resulted in lighter plants but all were fertile. Among these three lots best yields in number and weight of seed were obtained from plants on 16-hour photoperiod.

7. The differences resulting from the photoperiodic treatments have been attributed to the influence upon the plants of low-intensity supplemental light.

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EFFECT OF NUTRITION AND AGE UPON RATE OF DEVELOPMENT OF TERMINAL STAMINATE INFLORESCENCES OF XANTHIUM PENNSYLVANICUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 533

AUBREY W. NAYLOR

(WITH THREE FIGURES)

Introduction

Attention has recently been called to the effect of a number of environmental factors on floral initiation in soybean (*Soja max*) and cocklebur (*Xanthium pennsylvanicum*). These studies have been, for the most part, designed to determine the effect of (*a*) photoinductive cycles longer than 24 hours (**16**); (*b*) high and low temperatures during the photoinductive cycles (**2**); and (*c*) light intensity previous to, during, and after the photoinductive cycle—on floral initiation without regard to subsequent development. Some data, however, are given concerning the rate of development subsequent to initiation (**6, 10, 13, 16**). In practically all cases, however, the photoperiods employed were considerably above the critical.

Although some work has been done to determine the effects of different types of environments on the critical day length, few data are available on rate of development subsequent to photoinductive cycles near the critical. Temperature during the dark period, humidity, and light intensity were each found by LONG (**10**) to be important factors in altering the critical day length of *X. pennsylvanicum*. Also in *Rudbeckia bicolor*, a long-day plant, increase in temperature from 25° to 28.3° C. resulted in decrease in the critical day length (**17**).

The present work deals with the effect of nutrition and relative age of leaf tissue on the initiation and rate of development of floral primordia subsequent to treatments with photoinductive cycles at the critical and above.

Material and methods

The experiments reported here were performed in the University of Chicago greenhouses during the spring and summer of 1939. Burs of *Xanthium pennsylvanicum* were collected in Chicago during the autumn of 1938 and stored out-of-doors until early in 1939. In February they were dried and kept inside until shortly before use. Then the seeds were removed from the fruit, nicked slightly to rupture the seed coats, and planted. In all cases the seeds were germinated in flats on well-lighted greenhouse benches where supplementary illumination of about 100 foot-candles was supplied from shortly before sunset to 2:00 A.M. by Mazda filament lamps. Young seedlings, after attaining 4–6 inches in height (except in

the nutrition experiment), were transplanted to $3\frac{1}{2}$-inch clay pots. In the nutrition experiment the seeds were germinated in sand and the plants transferred to small glazed crocks filled with sterile sand, two plants being used in each crock. After transplanting, all plants were maintained on long photoperiods (18–20 hours of light and 6–4 hours of dark) until experimental treatment was begun.

Inasmuch as the soil was rapidly leached by the application of tap water twice daily, it was found necessary to add a complete nutrient solution at frequent intervals to prevent the plants from showing deficiency symptoms. This nutrient also served as the basic solution (table 1) for the nutrition experiment.

TABLE 1

CONCENTRATION OF CONSTITUENTS OF COMPLETE AND DEFICIENT SOLUTIONS AS APPLIED TO THE PLANTS

SOLUTION	CONSTITUENTS OF SOLUTIONS
Complete nutrient	$Ca(NO_3)_2$ . . . . . 0.006 molar
	KH_2PO_4 . . . . . 0.0045 molar
	$MgSO_4$ . . . . . 0.0045 molar
	$B(H_3BO_3)$ . . . . . 0.5 p.p.m.
	Fe as ferric citrate . . . . 1 cc. 0.5% solution/liter
	$MnCl_2$ . . . . . 0.5 p.p.m.
	$CuCl_2$ . . . . . 0.125 p.p.m.
	$ZnCl_2$ . . . . . 0.5 p.p.m.
Minus nitrogen	$CaCl_2$ substituted for $Ca(NO_3)_2$
Minus potassium	NaH_2PO_4 substituted for KH_2PO_4
Minus phosphorus	KCl substituted for KH_2PO_4

Stock plants were dissected at frequent intervals to make certain that all were vegetative. At the time of harvest the terminal inflorescence primordia were arbitrarily classified into seven different stages of development, varying from the vegetative bud to barely submacroscopic inflorescences. This classification is similar to that used by MANN (11).

Procedure and results

EFFECT OF MINERAL NUTRITION AND LENGTH OF DARK PERIOD UPON INITIATION AND DEVELOPMENT OF INFLORESCENCE PRIMORDIA

Previous experiments (7) have shown that under ordinary greenhouse conditions of summer, *X. pennsylvanicum* requires at least $8\frac{1}{2}$ hours of darkness out of 24 before it will initiate floral primordia. Inasmuch as mineral nutrition exerts considerable effect on development subsequent to initiation, this experiment was designed to determine the effect of low nitrogen, low potassium, and low phosphorus on ability to initiate floral primordia and the rate of their subsequent development.

On June 8, seeds were planted in washed quartz sand which was watered periodically with nutrient solution. After germination, when the seedlings were ex-

panding their first leaves (June 23), selections were made for uniformity in vigor and development. Two of these young plants were placed in each small crock and the sand thoroughly watered. Twenty crocks were placed in each of the small

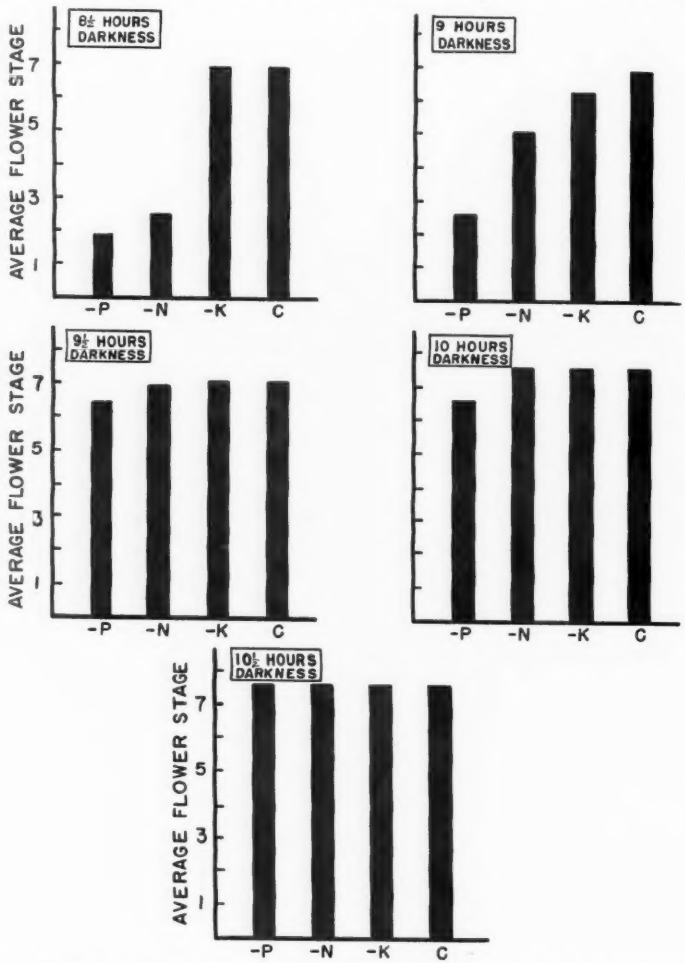


FIG. 1.—Effect of mineral nutrition and number of hours of darkness out of 24 on rate of terminal inflorescence development.

framework chambers where conditions of long photoperiod were maintained. Distilled water was added for 3 successive days. At that time the plants appeared to be in excellent condition.

On June 26, four different types of nutrient solutions were given to each of four different groups of plants in each of the chambers. The four groups (ten plants

each) received complete, −N, −K, and −P solutions until the end of the experiment. Characteristic deficiency symptoms were beginning to develop when short photoperiod treatment was begun. Starting with July 12, dark periods of the following duration were given in the chambers: 8, 8½, 9, 9½, 10, 10½, and 16 hours. All plants received 8 hours of daylight. The plants were then darkened and supplied in six of the compartments with Mazda filament light of approximately 100 foot-candles at the leaf surface, the length of the light period being automatically controlled by time clocks. In the seventh compartment no supplementary light was used. The plants were harvested 17 days later when floral primordia were macroscopic in the 8-hour photoperiod plus 16–hour dark period group.

The results of this experiment are shown in figure 1. No figures are given for the unit which provided only 8 hours of darkness because all plants were strictly vegetative. Of the units which received 9½ or more hours of darkness, none had any strictly vegetative plants; and there was only one among the 9-hour group.

Plants which received complete nutrients and −K had almost completely formed inflorescences if they were subjected to 8½ or more hours of darkness out of 24. The plants which developed next in rapidity were those low in nitrate; inflorescence primordia of plants deficient in nitrogen, however, developed as rapidly as any others when they had 9½ or more hours of darkness. Those plants showing the slowest rate of development in relation to length of the dark period were those low in phosphorus. Even these plants produced flower primordia as completely developed as under any other condition, provided the length of the dark period was 10 or more hours out of 24. Thus although mineral nutrition apparently does not alter the critical day length of *X. pennsylvanicum*, it does alter to varying degrees—depending upon the number of hours of darkness received—the rate of development of the staminate inflorescences.

Critical day length of old and young leaves

In a preliminary experiment with 2-month-old plants, none of the leaves were removed. In this instance the leaves selected for experimentation were of two kinds. Half the plants had their first fully expanded leaf, while the other half had one of their very slightly yellowing leaves selected for experimentation. Tops of cardboard cans, painted on the outside with aluminum paint and on the inside with a flat black paint, were slit to the center where a larger opening was made to fit the petiole. The can top was then inserted over the desired leaf of any one plant and tacked firmly to a wooden arm projecting from a right-angle stand. All cut openings were then sealed with adhesive tape or molding clay. The remainder of the can was placed over the leaf blades to provide dark periods varying from 8 to 12 hours out of 24. This single-leaf treatment was continued for 4 days, while the remainder of the plant was on long photoperiod. Subsequently the entire plants received long photoperiods. Dissections made of the terminal bud 5

weeks later indicated merely developmental trends but did not show great uniformity in any one treatment. The indications, however, were that younger leaves could bring about floral induction with shorter dark periods than could older leaves. Also, plants having their young leaves subjected to short photoinductive cycles flowered more vigorously than those having their older leaves so treated.

From this and other experiments (**3, 7**) it appears possible that the presence of leaves other than those being induced may exert some effect upon the degree of floral response, and that inequalities in area and number of leaves could bring about lack of uniformity in response. Accordingly, an experiment was designed in which all leaves except the one being tested were removed from the plant.

The plants used in this experiment differed from the first in that they were 7 weeks old at the beginning of treatment. Photoperiodic treatment was secured by means of the small chamber and time-clock control system previously described. Two types of controls were employed to determine the effect of different photoperiods on leaf primordia and stems. These consisted of completely defoliated plants in each of the seven chambers and plants maintained on long photoperiod continuously. The different groups of plants received 8 hours of natural daylight plus sufficient Mazda filament illumination to provide photoperiods of 9, 13½, 14, 14½, 15, 15½, and 16 hours out of 24. Treatment was continued for 4 days, the plants then being transferred to long photoperiod, where they remained throughout the experiment.

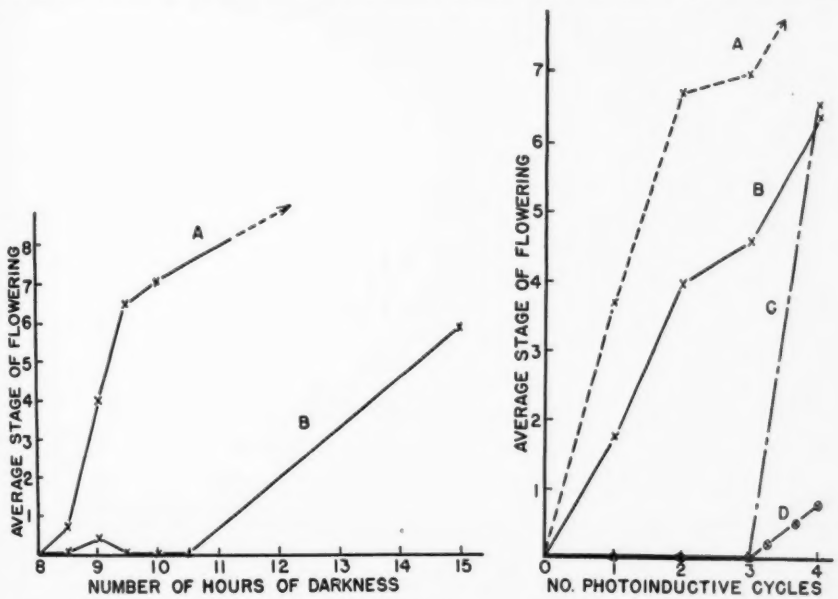
Data given in figure 2 show that the critical dark period for old leaves lies between 10½ and 15 hours, whereas for young leaves it was 9 hours. Thus the young leaves appeared to be much more sensitive to photoinductive treatment than the older ones. Rate of development subsequent to initiation, however, seemed to be directly related to length of the dark period. Figure 2 shows that as the dark-period treatment increased the average stage of inflorescence development at the time of harvest was more advanced.

A possible explanation for the flowering of two plants which received 9 hours of darkness is that the rapidly expanding leaf primordia enlarged sufficiently during experimental treatment to become photoperiodically sensitive. Because the leaves sometimes expanded sufficiently in a day's time to become photoperiodically sensitive, it was necessary to remove the older primordia daily.

Effect of age of leaves on number of photoinductive cycles required for induction

In the first experiment, performed to determine the number of photoinductive cycles a single leaf required in order to bring about flowering, not more than one of the leaves was removed from the plant. Inasmuch as the cardboard-can method previously described was being used, it was occasionally necessary to remove one

leaf in order to prepare the expanded leaf for short photoperiod treatment. No strict attempt was made to use either very young or very old leaves; those selected were about midway between the two ages. The twenty plants thus prepared were subjected to four different treatments. Groups of five plants each received 1, 2, 3, or 4 photoinductive cycles consisting of 9 hours of light and 15 hours of darkness. After these treatments the entire plants were left on long photoperiod



FIGS. 2, 3.—Fig. 2 (left), effect of age of leaves of plants which had all except (*A*) single young leaves and (*B*) single old leaves removed on rate of development and number of hours of darkness out of 24 required to bring about floral initiation. Fig. 3 (right), rate of floral development of plants which had (*A*) all except one young leaf removed before the experiment began; (*B*) single young leaf exposed to short photoperiod, no leaves being removed; (*C*) all except one old leaf removed before the experiment began; (*D*) single old leaf exposed to short photoperiod, no leaves being removed.

until one month later. At that time dissection of the terminal buds showed that with one exception the only plants having inflorescence primordia were those which had a single leaf exposed to four photoinductive cycles.

This experiment was followed on June 14 by another in which all the leaves except the first expanded one were removed. Twenty plants were so treated; five which served as controls had all leaves removed. All plants were placed on a truck to receive 9 hours of light followed by 15 hours of darkness. At daily intervals during the treatment five plants with single leaves were removed from the truck and returned to long photoperiod benches. The defoliated controls, to-

gether with the remaining five experimental single-leaved plants, were returned to long photoperiod benches following the fourth photoinductive cycle. During the short photoperiod treatment, expanding leaf primordia were not permitted to enlarge beyond 1 sq. cm. before being removed.

Twenty-six days after the start of the experiment dissections were made. At that time it was found that three of five plants receiving only one photoinductive cycle had flower primordia; four of five plants receiving two photoinductive cycles had flower primordia; all other plants receiving a greater number of photoinductive cycles were flowering.

Because of the apparent differences in behavior of the different aged leaves just noted, an experiment was performed, first to determine the number of days required for induction of old and young leaves when all leaves were present and second to determine the number of days required for induction when only the individual leaves being tested remained on the plants.

For the first part of the experiment eighty uniform vegetative plants were selected and divided into two equal groups. The first fully expanded leaf was selected in one of these groups and old but not yellowing leaves were selected in the second. Triple-thickness black sateen cloth bags were placed over individual leaves at 5:00 P.M. and removed at 8:00 A.M. In order that the bags might not injure the leaves, a thin cane was placed in each pot at such an angle and trimmed to such a length that when the bag was placed over it the open end of the bag reached the base of the petiole. As further precaution against injury, and for stability, the petiole was bound to the cane. After placing the bag over the leaf and end of the cane, its open end was closed around the petiole with a rubber band. Photoinductive cycles were supplied units of ten plants each for 1, 2, 3, or 4 days in both of the age groups. After each unit had received the specified number of photoinductive cycles, the entire plant was maintained on long photoperiod.

Thirty-three days after the experiment was begun dissections were made. The plants which had single young leaves exposed to photoinductive cycles for only one day showed very slight primordial development; those which received a greater number of days of induction showed progressively advanced stages (fig. 3). For the most part, the plants which had had single old leaves exposed to photoinductive cycles were vegetative. None of the plants which had leaves receiving 1, 2, or 3 days of treatment showed any indication of change from the vegetative state, and of those receiving 4 days of treatment only one was definitely producing flower primordia while five others had growing points only slightly changed from the vegetative condition (fig. 3).

In the second experiment, 120 plants were selected for uniformity and placed on a short-photoperiod truck. Of these plants, forty were defoliated except for

the first expanded leaf; forty others were defoliated except for an old leaf; controls consisted of twenty completely defoliated and twenty undefoliated plants. Units of ten plants from each of the single-leaved groups were removed to long photoperiod conditions following each photoinductive cycle. Ten of the undefoliated controls were removed after the second and fourth cycles.

When the plants were harvested 39 days after the experiment began, four of the plants which had a single young leaf exposed to one photoinductive cycle had slightly submacroscopic flowers, four others showed younger stages, and two were vegetative. Those whose leaves received two and three photoinductive cycles all had slightly submacroscopic inflorescences, while those receiving four were macroscopic (fig. 3). In contrast, of those plants which had only one old leaf exposed to photoinductive cycles, none receiving one or two cycles produced flower primordia, only one of ten receiving three cycles showed flower primordia, while all those receiving four cycles had nearly macroscopic inflorescences. All the defoliated controls were vegetative; but of those which had not been defoliated half those receiving one photoinductive cycle had visible inflorescences, the others being almost macroscopic. All those which received two photoinductive cycles had well-developed inflorescences.

Discussion

Considerable work has been done to determine whether flower formation is definitely associated with any one of the mineral elements known to be important in other phases of development. As a result of work with monoecious and dioecious plants, it has been shown that nutrition does affect the degree and direction of floral expression. TIBEAU (**19**) found that hemp responded to high nitrogen conditions by producing pistillate flowers, while if only a small amount of nitrogen was available his plants were entirely staminate. There is also evidence that highly localized concentrations of nitrogen exert marked effects on development of staminate and pistillate structures of tomato (**8**), corn, cucumber, and watermelon (**12, 20, 18**). In addition, NEIDLE (**14**) has shown that the proportion of staminate and carpellate heads of *X. pennsylvanicum* is dependent upon the carbohydrate-nitrogen relation of the plant and the number of photoinductive cycles received. That other elements may exert some influence upon the development of flowers has been suggested by COMBES (**4**). There is, however, little evidence to support the theory that floral initiation in photoperiodically sensitive plants results directly from conditions of mineral nutrition. Yet the direction and degree of subsequent development are in most instances markedly affected. This is true also for the tomato, a day-neutral plant, which initiates floral primordia under extremely wide ranges of carbohydrate and nitrogen supply, although subsequent floral and fruit development are sharply limited by the same factors (**9**).

In the work reported here, it has been shown that even though there was a very low supply of nitrogen, potassium, or phosphorus available, the critical day length of *X. pennsylvanicum* was unaffected. The rate of development of these primordia, especially near the critical photoperiod, depended to a considerable degree upon both phosphorus and nitrogen. The detrimental influence of low supply of these elements on rate of development was overcome, however, by increasing the length of the daily dark period. With increase in the dark period there was an increasing tendency for all primordia to develop at the same rate. Thus if optimum conditions of light and darkness (10 hours of light plus 14 hours of dark) are suddenly imposed, the effect is at least twofold: (*a*) an initiation of the flower-promoting stimulus and (*b*) mobilization of both food materials and nutrients at the terminal growing point, even in plants low in nitrogen, phosphorus, or potassium, in sufficient quantities for the rates of development to be approximately the same. NIGHTINGALE (**15**) has previously shown with tomatoes that, even if they are grown with a nitrogen-free solution, the amount of soluble nitrogen increases if the plants receive long dark periods. This indicates that there may be reutilization of nitrogenous compounds within the plant. Under conditions of increasing dark periods the amount of soluble nitrogen present at the growing tip of a nitrogen-deficient plant would be expected to increase, with the result that the flowers would grow more rapidly. While it may not necessarily be true that phosphorus- and potassium-containing complexes would under similar conditions of increasing darkness be reutilized, the increasing rate of growth of the cells at the apex when the dark period is increased indicates that this is true.

In addition to nutrition, the age and size of the plant may be effective factors in altering response to photoperiodic treatment. BORTHWICK and PARKER reported (**1**) that flower primordia may be produced at more nodes of older than younger soybeans up to the age of 6 weeks. Moreover the individual leaves possess varying capacity for bringing about the flowering response. Their subsequent investigations have shown that any expanded leaf on a Biloxi soybean plant subjected to photoperiodic stimulation is able to cause the formation of flowers at the growing points. In these experiments (**3**) they found that—on the basis of the number of flowers produced—the young leaf rapidly increases in ability to stimulate floral initiation until it is fully expanded. Subsequently its capacities to bring about such a response decrease.

While considerable information is available to indicate that growth-regulating substances are produced in relatively young tissues, there is little evidence to show the effects of aging of tissues on their production. Data presented here indicate that old leaf tissue does not bring about nearly as rapid production of the stimulus to floral initiation as do young leaves. There is also some evidence that the

presence of older leaf tissue may have an inhibitive effect upon the rate of development of inflorescence primordia.

It was found that if there are no intervening leaves between the leaf being induced and the stem-tip inflorescence, development proceeds rapidly. On the other hand, in the presence of leaf tissue other than that subjected to photoinductive treatment there is a sharp reduction in the rate of inflorescence development. These findings may be interpreted in at least two ways. It is possible that the flower-promoting substance is destroyed in the stem or leaves, but equally likely that there is a general movement of the stimulus into all leaf tissue, thus permitting little of the stimulus to reach the growing tip. If the stimulus is diverted into the leaf tissue and not destroyed, we should expect the axillary buds to develop into flower primordia. In *X. pennsylvanicum*, however, the terminal inflorescence develops even before the axillary bud in the axil of the induced leaf. Development of axillary buds usually occurs only after the terminal inflorescence is produced. Inflorescences are produced progressively downward from the stem tip (**13**). Usually the lowest axillary buds do not produce fertile flowers.

In these experiments no evidence was found to support the viewpoint of CHOLODNY (**5**) that the flower-promoting stimulus is autocatalytic in action. In every instance it was found that, up to a certain number of photoinductive cycles —depending upon the age of the leaves and the number of leaves present—the rate of development depended upon the number of photoinductive cycles given. HAMNER's work with soybean (**6**) demonstrated that the number of flowers produced was almost directly proportional to the number of photoinductive cycles given. Thus rate of development and amount of inflorescence development appear to be dependent upon the amount of stimulation given through photoinductive cycles rather than upon continuous autocatalytic formation of the flower-promoting stimulus subsequent to its initiation.

Summary

1. *Xanthium pennsylvanicum* plants low in phosphorus, potassium, and nitrogen had the same critical day length as plants supplied with a nutrient solution containing all these elements.

2. Rate of development of terminal inflorescences of −N, −K, and −P plants was considerably affected at photoperiods near the critical. Lack of phosphorus decreased the rate of development more than lack of either nitrogen or potassium; and lack of nitrogen resulted in slower development than lack of potassium.

3. With each half-hour increase in length of the dark period from $8\frac{1}{2}$ to $10\frac{1}{2}$ hours there was an increasing tendency for the plants low in phosphorus, nitrogen, or potassium to develop at the same rate as the plants grown with a nondeficient solution. Thus it appears that by increasing the length of the dark period

the detrimental effect of mineral deficiency on rate of development of floral primordia may be overcome.

4. Age of leaf tissue definitely affects the critical day length. The first fully expanded leaf had a critical dark period of 9 hours, whereas in the older leaves it was between $10\frac{1}{2}$ and 15 hours. Rate of development subsequent to initiation was dependent on the length of the dark period.

5. Single young leaves may bring about induction to flower if given only one photoinductive cycle, but the flowering response is more pronounced if more cycles are given. Four photoinductive cycles brought about as rapid rate in inflorescence development in the plants defoliated except for one leaf as two cycles in the undefoliated plants. Old leaves, on the other hand, required four photoinductive cycles to bring about a change from the vegetative state.

6. The data substantiate the theory that the presence of leaf tissue other than that being photoperiodically induced decreases the effectiveness of the flower-promoting stimulus in bringing about floral initiation and the subsequent rate of development.

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MORPHOLOGICAL STUDY OF AGAVE LECHUGUILLA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 534

ALVIN R. GROVE

(WITH THIRTY-EIGHT FIGURES)

Introduction

STENAR (6) has compiled much of the literature of the Amaryllidaceae published previous to 1925. His discussion of the Agavoideae is brief, and much of his information is based on ERNST's work on *Fourcroya cubensis*. SCHAFFNER (3) and OSTERHOUT (2) have reported some work on pollen development, with special emphasis on the cytological investigation of microspore mother cell divisions and chromosome counts. SCHNARF (5) discusses in some detail the reproductive habits of some genera of the Amaryllidaceae, but statements concerning the genus *Agave* are meager and based again in large part on ERNST's work on *Fourcroya*. SCHLIMBACH (4), in discussing the Amaryllidaceae, makes general reference to the family but does not present details concerning *Agave*. GOEBEL (1) makes several short references to this genus, speaking in particular of its hollow style and the development of a multilayered endothecium in *A. americana*.

The research reported here includes studies of the origin and development of the floral organs and their relation to the axis and bracts which become involved in such development; the vascular anatomy of the flower; the development of the ovule; megasporogenesis and megagametogenesis; and the development of the anther. No effort has been made to count the chromosomes or to make a cytological investigation of the nuclear divisions. The present investigation started with actual flower formation, but much growth of the flowering axis has already preceded this stage. Elongation of the axis from the center of a basal rosette of foliar leaves is the first indication of flowering. This stalk elongates rapidly and may reach a height of many feet before recognizable flowers appear. The material studied was collected from the tip of this axis after its elongation had taken place.

Material and methods

Material of *Agave lechuguilla* Torr. was collected in the field near El Paso, Texas, May 7, 1939, and June 1, 1940, and also at Alpine, Texas, May 5, 1939. Flowers and developing fruits were dissected from healthy plants and were killed and fixed in Sax's modification of Navashin's solution. The material was separated into approximately twenty age groups, from tips to oldest fruits, and later sectioning was done on random samples from these groups. Treatment was according

to the paraffin method, and sectioning was at 3–15 μ. Flemming's triple stain and light green and safranin were employed. At the time that material was fixed for sectioning, other material was killed in formalin-acetic-alcohol and used for gross study.

Observations

Origin and development of flower

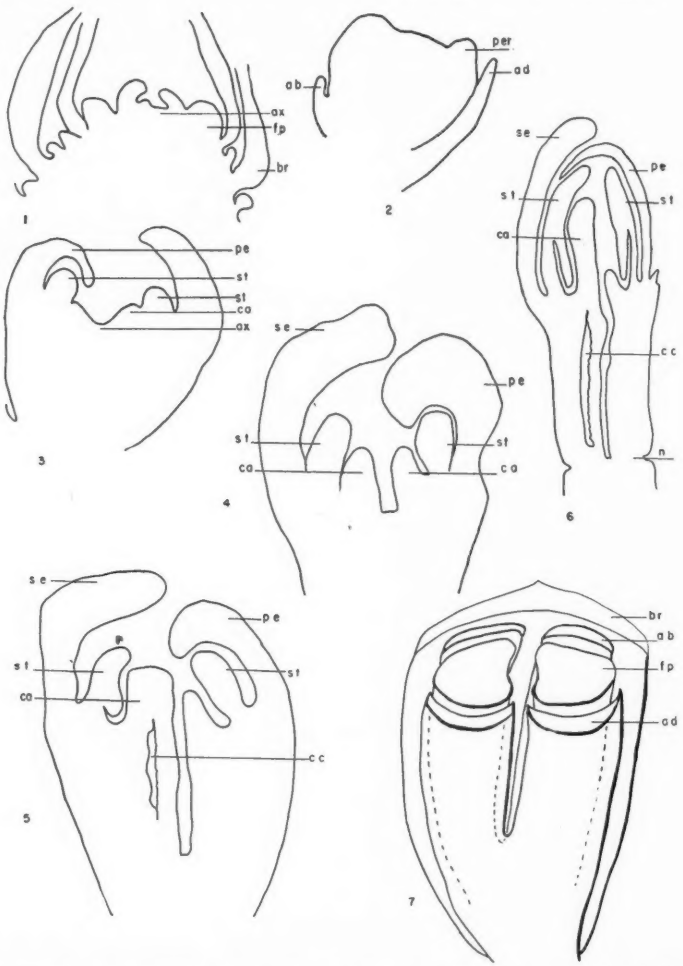
The first evidence of floral development is the appearance of a single branch primordium in the axil of each bract, referred to here as the outer bract, diverging from the main axis of the plant (figs. 1, 7). Before appreciable elongation of this branch primordium has taken place, two bracts, referred to here as the adaxial flower bracts, arise near its base and toward the axis. As development of these bracts takes place, their bases elongate conjointly and at first only their tips are separate. Later, after the flowers themselves are well developed, the conjoint base splits so that apparently two completely separate bracts are present, one on the adaxial side of each flower branch. Before any elongation of the first branch primordium has taken place a second one arises in the axil of the bract on the first branch primordium. In a cross section of the young flowering axis these two primordia lie inside the outer bract and outside the conjoint base of the two developing adaxial bracts (fig. 7). The origin of the first and second branch primordia and the two adaxial bracts can be determined from cross sections in which the bases of all four structures are common. Later, as elongation takes place, each can be seen as a separate structure near its tip. The first and second branch primordia are completely covered with an epidermis, as are both adaxial bracts. In addition to the structures already mentioned, each flower primordium bears a bract on its outer face, the abaxial bract. These bracts develop later than the adaxial bracts, and flowers may or may not develop in their axils. This development usually leads to the formation of two flowers, one differentiated at the tip of the first branch primordium and one at the tip of the second. Not always, however, does development stop with two flowers, and cases can be found where three or even four ultimately develop. Such a branch system of flower origin would seem the normal behavior for the plant and accounts for the origin and variation in number of flowers present.

Perianth and sepals

The perianth arises first as a ring on the periphery of the flower primordia. Not long after, the three sepals arise from this as separate primordia. At this time there is much lateral growth of the end of the branch, which causes it to widen and flatten (fig. 2). Subsequent elongation of the sepal primordia (figs. 3–6) leads to three elongated and flattened sepals which are widened at their base and which in the mature flower overlap the petals to some extent.

PETALS

The petal primordia, three in number, arise next in order and lie just inside the ring of sepal primordia and alternate with them (fig. 3). They eventually elongate



FIGS. 1–7.—Figs. 1–6, successive development of floral parts. Fig. 7, reconstruction showing relation of bracts to flower primordia; axis removed. (*ax*, axis; *fp*, flower primordia; *br*, outer bract; *ab*, abaxial bract; *se*, sepal; *pe*, petal; *ca*, carpel; *st*, stamen; *cc*, carpellary cavity; *n*, node.)

and are sepaloid in appearance (figs. 4–6), contain chlorophyll, and are not showy. In shape they are similar to the sepals and approximate them in size. In the mature flower their edges are overlapped by alternating edges of the sepals.

Stamens

Two rings of three stamens each are next in origin. The outer ring of three lies opposite the three sepals and the inner ring of three within and opposite the three petals. The primordia arise as more or less dome-shaped protuberances, and all six stamens develop simultaneously in two cycles (fig. 3). At the time the stamens originate the sepals are already well developed and the petals are rapidly elongating. In later stages of development the anther arises as a terminal expansion of the stamen. Its growth is parallel to the filament, and in the mature condition the filament is attached at about the center of the elongated anther, being versatile. When the flower opens at time of pollination the anther flips up, its median attachment to the filament operating as a hinge (fig. 6).

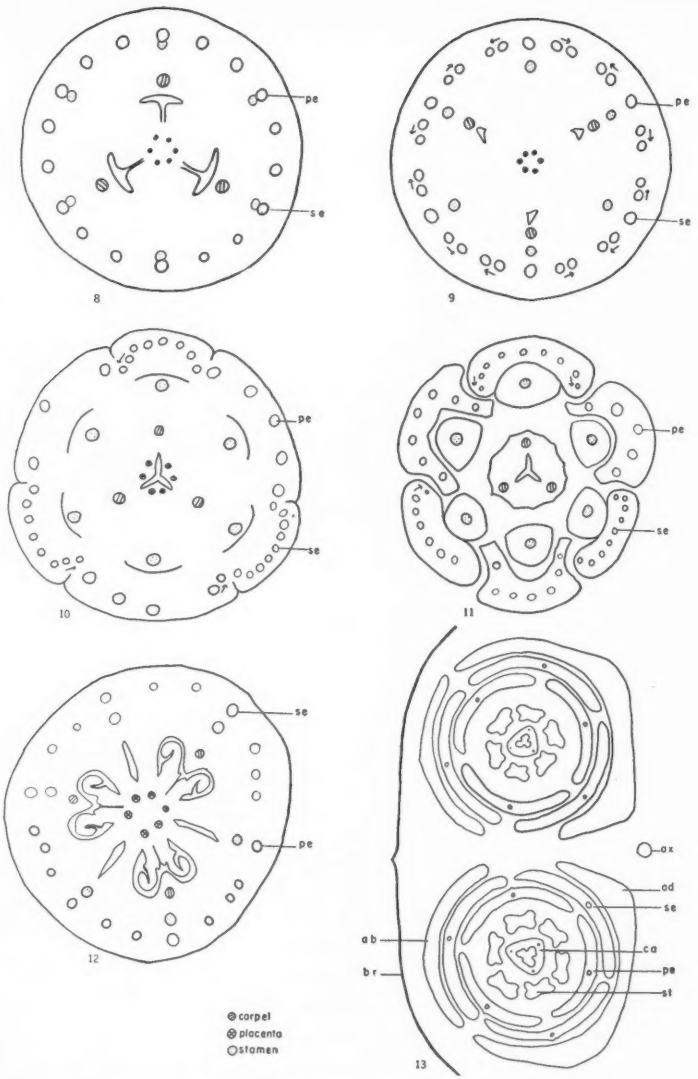
Carpels

The three carpel primordia arise last and opposite the sepals, and, like the stamens, they are at first more or less dome shaped (fig. 3). At this stage in ontogeny the growth of the end of the axis is inhibited, but all the conjoint flower parts continue to elongate and enlarge above it, so that in the young flower a canal is left in the center of the developing ovary. However, the conjoint development of the three carpels forms a common central tissue which completely fills this central canal in the mature flower (fig. 12) and leads to the formation of a compound ovary with three separate and completely inclosed carpellary cavities. Zonal elongation of the conjoint bases of the three carpels completes the formation of the compound pistil. The tips of the three carpels continue above the ovary as a compound style, which is three lobed and hollow. As zonal elongation of the three carpels takes place, zonal elongation of the undiverged bases of the sepals, petals, and stamens also occurs. This conjoint tissue completely surrounds and is indistinguishable from the carpels (fig. 12).

The general development just described is one of acropetal succession, starting with sepals and progressing inward toward the center of the flower through petal, stamen, and carpel.

Vascular anatomy of flower

A node at the bottom of the ovary makes it difficult to trace the vascular supply of the flower down through the flower branch (fig. 6). In a very young flower, before differentiation of the majority of the procambial strands, it is possible to trace a certain amount of vascular tissue through the node, but since all traces are not differentiated, the lack of obvious nodal ramifications complicates the interpretation rather than simplifies it. For this reason no attempt has been made in the drawings to trace the vascular supply through the node; only the bundles above it after vascular tissue differentiation has taken place have been recorded (figs. 8–13). In no instance have drawings been included at the tips of sepals, petals, etc.



FIGS. 8–13.—Figs. 8–11, successive cross sections at representative levels. Fig. 12, cross section of older ovary showing relation of ovules. Fig. 13, diagrammatic cross section of two flowers, showing orientation of floral members. (*se*, sepal; *pe*, petal; *ax*, axis; *ad*, adaxial bract; *ab*, abaxial bract; *br*, outer bract; *ca*, carpel; *st*, stamen.)

At the base of the ovary there is visible a median bundle for each sepal and each petal. In addition there are twelve lateral bundles, one on either side of each median sepal and petal bundle. On the inner side of each median sepal and petal bundle is a stamen bundle. In most cases at this level this bundle is so closely appressed that it is difficult to distinguish it. Three dorsal carpel bundles are present at this level and remain unchanged throughout the upper extent of the flower, passing into the style at the top of the ovary. Six placental bundles are present, but at this stage show little differentiation (fig. 8).

Examination of cross sections of the flower at higher levels shows that the median bundle of each sepal passes into the sepal without dividing. Each lateral bundle is divided to give rise to one toward the edge of the sepal. The bundles formed by this first division divide, and the derived bundles here divide once. This leads to the usual condition of nine bundles present in the sepal after its divergence at the top of the hypanthium (fig. 11). The petal bundles show the same kind of development, except that two divergences usually occur and seven bundles rather than nine is the usual number present in the petal (fig. 11). The stamen bundles, as already mentioned, lie inside of and are tightly appressed to each median sepal and petal bundle (fig. 8). At the top of the hypanthium the stamen bundles pass directly up into the stamens (fig. 11).

The vascular plan above the node is a simple one, and indicates that the hypanthium consists of the undiverged bases of sepals, petals, and stamens, as shown by their vascular anatomy.

Ovule development

The nucelli arise from the lateral edges of each of the three carpels. As growth continues the nucellus elongates, and with growth more rapid on the outer face the nucellus gradually turns through an arc of nearly 180° to become anatropous. Two integuments are developed from the base of the nucellus and ultimately overgrow its end to form the micropyle. The outer integument is fully developed only on the outer face of the ovule and is present only as a ring on the funicular side.

Before any appreciable curvature of the nucellus, and previous to the origin of the inner integument, the archesporial cell is differentiated. The megaspore stages are numerous when the nucellus has passed through about a 90° arc, and one integument is developed—with the second starting in some instances. Megagametophyte stages occur when the ovule has assumed its complete anatropous position and both inner and outer integuments are well developed.

Megasporogenesis

A single hypodermal cell enlarges from the sporophytic tissue of the nucellus. It can readily be identified by its position, immediately beneath the epidermis; its increased size and denser cytoplasm; and its enlarged nucleus. It is differentiated

before the appearance of the inner integument and before any appreciable growth of the nucellus has taken place (fig. 14). The first and only division of the archesporial cell is periclinal, resulting in the primary sporogenous cell on the inside and the primary parietal cell on the outside (fig. 15). The primary parietal cell is divided in turn by an anticlinal division (fig. 16) and eventually gives rise to a single parietal layer (figs. 17–20). The parietal tissue persists until the megagametophyte starts to enlarge, at which time it is crushed.

The primary sporogenous cell enlarges directly into the megaspore mother cell (fig. 17). Two divisions of this cell and its derivatives follow in rapid succession, and a linear tetrad of four megaspores is formed (figs. 18, 19). Not until both divisions are completed is there evidence of wall formation, but following the second division walls come in to form four cells (fig. 20). Although chromosome counts were not made, it is assumed that these two divisions involve reduction of the chromosomes from the diploid to the haploid number. Of the linear tetrad so produced the megaspore toward the chalazal end of the ovule survives and the remaining three disintegrate (fig. 20). This results in an enlarging uninucleate cell which becomes directly the young megagametophyte (fig. 23).

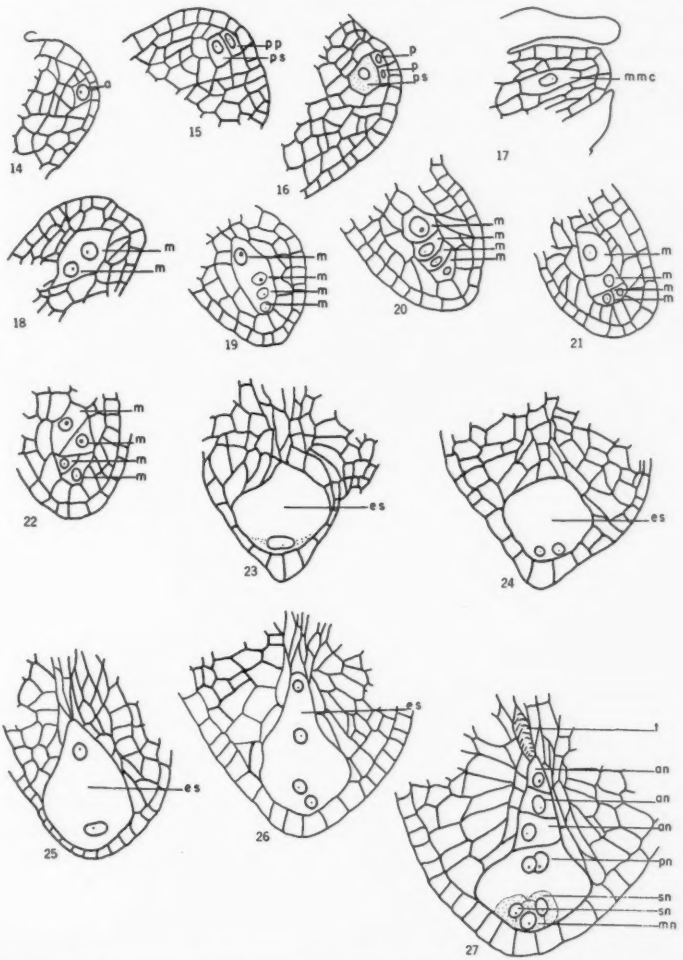
Variation in megaspore arrangement

In addition to the usual linear tetrad arrangement of the four megaspores, two variations were noted rather commonly. One variation is essentially the same as the linear tetrad, except that the walls between the megaspores come in at angles to one another instead of parallel (fig. 22). A second variation shows the wall between the two micropylar nuclei at right angles to the wall between the other two toward the chalazal end (fig. 21). This is similar to a condition already recorded for *Haemanthus katharinae* and *Bomarea caldasii* (**6**).

Megagametophyte development

The single nucleus of the enlarging young megagametophyte is located near the micropylar end (fig. 23). It divides to give rise to two nuclei (fig. 24) and one nucleus, then migrates toward the chalazal end (fig. 25). The cytoplasm between these two nuclei, one the primary chalazal and the other the primary micropylar, becomes highly vacuolated as enlargement occurs. Each of these two primary nuclei divides to give rise to a four-nucleate megagametophyte (fig. 26). Division of the primary micropylar nucleus precedes that of the primary chalazal nucleus. Each of the four nuclei now present undergoes one more division, giving rise to eight. Of the four at the micropylar end of the megagametophyte, one develops as the egg, two as synergids, and one as a polar nucleus. Three of the four nuclei at the chalazal end become nuclei antipodal cells arranged in linear manner and one a polar nucleus. The two polar nuclei migrate toward the center of the megagame-

tophyte and together make the primary endosperm nucleus, fusion not taking place until fertilization or soon thereafter. The egg and each synergid have a defi-



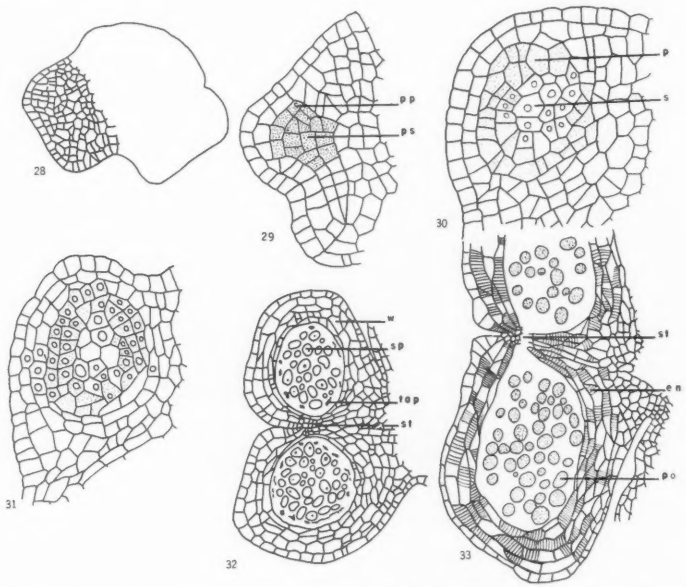
FIGS. 14–27.—Megasporogenesis and megagametogenesis. (*a*, archesporial cell; *pp*, primary parietal; *ps*, primary sporogenous; *p*, parietal; *mmc*, megaspore mother cell; *m*, megaspore; *es*, embryo sac; *t*, tracheid; *an*, antipodal nucleus; *pn*, polar nuclei; *sn*, synergid; *mn*, megagamete nucleus.)

nite wall and a fairly large amount of cytoplasm. The cell containing the two primary polar nuclei is much larger than any of the others (fig. 27). The cytoplasm of the two synergid cells cups slightly around the egg cell, which takes a central position between the two synergid cells.

Instead of the vascular tissue ending at the base of the chalaza, tracheids are often differentiated in the tissue between the chalaza and the megagametophyte itself. Apparently there are only tracheids and there is no indication that phloem elements are present also. This condition is rare in plants (fig. 27).

MICROSPORES AND MICROGAMETOPHYTE

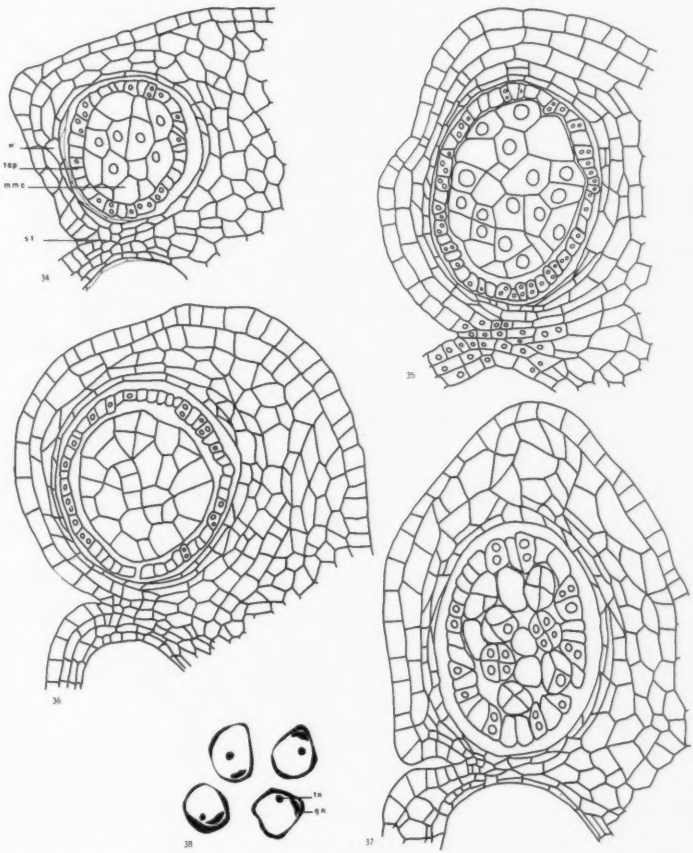
The anther, when mature, is terminal and versatile. As its development proceeds, four distinct lobes are formed. At first, all the cells of these lobes, exclusive of the epidermis, are similar in size, in density of cytoplasm, and in general appearance (fig. 28).



FIGS. 28–33.—Anther development. Figs. 28–31, early development of parietal and sporogenous tissue. Fig. 32, microspore separation. Fig. 33, mature anther. (*ps*, primary sporogenous; *pp*, primary parietal; *w*, wall; *st*, stomium; *en*, endothecium; *sp*, spores; *po*, pollen; *tap*, tapetum; *s*, sporogenous; *p*, parietal.)

The archesporium differentiates as four groups of cells from hypodermal tissue, one in each lobe of the anther. The archesporium is not easily distinguished at first, but by the time of periclinal division of these cells in the formation of primary parietal and primary sporogenous tissue it is easily distinguished (fig. 29). The primary parietal tissue thus formed undergoes several other periclinal and random anticlinal divisions in the formation of wall tissue which surrounds the more or less centrally located sporogenous mass (figs. 29–31). The innermost layer of parietal cells differentiates as tapetum. The cells of the tapetal layer are small at first but

gradually enlarge, and by the time microspore mother cells are recognized many of the tapetal cells are binucleate. Although the tapetum is fully formed by the time microspore mother cells are differentiated, further enlargement of the tapetal



FIGS. 34–38.—Anther development. Fig. 34, microspore mother cells. Fig. 35, at time of first reduction division. Fig. 36, at time of second reduction division. Fig. 37, separation of spores. Fig. 38, mature pollen. (*mmc*, microspore mother cell; *tap*, tapetum; *w*, wall; *st*, stomium; *tn*, tube nucleus; *gn*, generative nucleus.)

cells does take place and its greatest development is simultaneous with the formation of microspores. From this period on the tapetum disappears and the microspores enlarge and develop. In some instances at least, disintegration of the tapetum supplies a nutritive mass which surrounds many of the newly separated spores. The tapetum and its remnants continue for a long time during spore de-

velopment, but when mature pollen grains are found it has entirely disappeared (figs. 34–37).

The remaining wall cells derived from the parietal tissue stay more or less intact, although there may be some disintegration of the inner layer as the tapetum enlarges. When the anther matures the remaining wall tissue is characterized by the formation of a well-defined endothecium, which involves all but the epidermal layer of wall tissue (fig. 33).

The primary sporogenous tissue is formed by division of the archesporium. These primary sporogenous cells may undergo one or more divisions and the resulting cells enlarge and function as microspore mother cells (figs. 30, 34). The microspore mother cell undergoes two successive divisions, the reduction divisions, and wall formation follows each immediately, forming four microspores (figs. 34–36). Following the second division the microspores separate, usually in groups of four cells, following the pattern of the original microspore mother cells; but these groups soon separate, the individual spores becoming independent. They are at first thin walled and uninucleate and may in part be surrounded with a cytoplasmic mass from the disintegrating tapetal cells (figs. 32, 37). As the microspores mature their wall continues to thicken, and division of the nucleus results in the formation of a two-nucleate microgametophyte. The tube cell is large and occupies the greater part of the pollen grain. In cross sections of mature pollen grains one thin place is always recognized in the wall surface surrounding the tube cell.

The generative nucleus is vermiform and its cell is considerably smaller and less regular in outline than the tube cell (fig. 38). Stomium structure is traced in figures 28–37. As the anther matures, with pollen grains and endothecia differentiation, the stomium breaks with little decomposition and the locular spaces of the two adjoining pollen groups become one (fig. 33).

Summary

1. Origin of the floral organs of *Agave lechuguilla* is acropetal, sepals appearing first and carpels last.

2. The flower is epigynous. Its vascular anatomy is simple, being a direct divergence of vascular bundles into the separate organs. All vascular bundles ending in either sepal or petal structures are derived from bundles differentiated early in connection with these floral parts.

3. The ovule is anatropous and has two integuments. Placentation is axial.

4. Megasporogenesis starts with differentiation of a single hypodermal archesporial cell and ends with formation of a tetrad of megaspores arranged usually in linear fashion. Two variations in megaspore arrangement were noted.

5. Periclinal division of the archesporial cell gives rise to a primary parietal cell and to a single primary sporogenous cell which enlarges and becomes the megaspore mother cell. Development of the megagametophyte follows the so-called normal type.

6. A single parietal layer is present in the ovule, disintegrating when the megagametophyte enlarges.

7. The vascular tissue of the ovule does not stop at the base of the chalaza but tracheids penetrate the nucellus to the megagametophyte.

8. Anther development follows the common method. The archesporium consists of a group of cells. Periclinal divisions give rise to the primary parietal and primary sporogenous cells. Continued periclinal and anticlinal divisions of the primary parietal cells form the wall tissue. The sporogenous cells enlarge and function as microspore mother cells. There are two successive divisions of the microspore mother cells, resulting in four microspores.

9. The tapetum is derived from the innermost layer of the parietal tissue.

10. The mature anther shows well-defined endothecia three or four cells in thickness and disintegration of the stomium.

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EFFECT OF VARIOUS GROWTH-REGULATING SUBSTANCES UPON SEVERAL SPECIES OF PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 535

DAPHNE B. SWARTZ

Introduction

Many recent investigations have been concerned with attempts to determine the functions of certain of the phytohormones. Notable among these substances are those—such as vitamin B_1 and nicotinic acid—which have been shown, in experiments with isolated embryos and excised roots, to be essential to root development (1, 8). These experiments indicate also an interdependence of these two substances. For example, BONNER (1, 2) found that while in certain instances nicotinic acid alone was not effective, when applied in addition to vitamin B_1 it intensified the response of the plant to the latter.

But whether these substances, under certain conditions, may become limiting factors to the extent that their external application will affect the total plant growth, is still a question. BONNER and GREEN (3) have reported increased dry weight of both roots and shoots, accompanying increased general vigor, when vitamin B_1 was supplied with nutrient solution to plants in sand cultures. In marked contrast to these results are those reported in the recent work of HAMNER (6). Under variously controlled conditions of photoperiod and nitrogen supply, plants supplied with vitamin B_1 showed no increased dry weight. GORHAM (5) also reports no increased frond multiplication in *Lemna* when vitamin B_1 was added to the solution in which it was growing.

Other substances, such as naphthaleneacetic acid and related synthetic compounds, though not yet known to be formed in plants, are known to increase the rate of root initiation when applied to stems, leaves, and plant parts other than roots, in general (9). These same substances, which initiate root formation, may fail to promote growth and even in high dilutions inhibit root growth (4). Somewhat at variance with this is the report of GORHAM (5) that concentrations of 0.25 and 0.10 mg. of naphthaleneacetic acid per 100 ml. of nutrient produced marked increase in the rate of frond multiplication in *Lemna*. It has also been well established that pronounced histological changes accompany root formation and other external changes induced by naphthaleneacetic acid (7). But the effect which these root-forming substances may have upon total plant growth (as indicated by dry weight), and the external conditions under which these effects may occur, are as yet largely undertermined.

The present investigation, except for a part of the preliminary experiment, attempted only to determine the effects resulting from the addition of various growth substances to plants supplied with a complete nutrient solution to the sand medium in which they were grown. Great numbers of plants were used to make statistical analysis of the results possible.

Investigation

Chrysanthemum

This experiment was conducted, in part, to determine whether the addition of a growth substance may in any measure compensate for the lack of an essential element in the nutrient solution.

On April 26, chrysanthemum cuttings which had been rooted and were of approximately uniform size were transplanted into 4-inch glazed pots with side drain-

TABLE 1

Dry weight* in grams of chrysanthemum plants

	I Complete nutrient		II Minus Mg nutrient		III Minus K nutrient		IV Minus P nutrient	
	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots
Control	4.72	2.11	0.81	0.17	0.4	0.16	0.17	0.21
Vitamin B_1 added	4.59	2.03	0.95	0.24	0.55	0.28	0.15	0.21
Naphthaleneacetic acid added	4.21	1.94	0.72	0.20	0.8	0.39	0.16	0.31

* Weights represent mean of fifteen plants grown under each treatment.

age. The pots had been previously filled with quartz sand and flushed with distilled water. They were supported on lath racks to prevent any contamination of the nutrient supply through drainage. Fifteen plants were provided for each of the four treatments (table 1), and the pots were placed at random on the greenhouse bench in rows of five each. During the 10 weeks which followed, the plants were supplied with nutrient solution, with added growth substances on alternate days, and with distilled water on intervening days. Once a week the pots were thoroughly flushed with distilled water. The complete nutrient solution used in this, as well as in the more extensive experiments which followed, was made up from the following 0.5*M* stock solutions. Of these there were added to 72 liters of distilled water: 864 ml. of $Ca(NO_3)_2$, 648 ml. of $MgSO_4$, and 648 ml. of KH_2PO_4. In the "minus" solutions, the following substitutions were made: NaH_2PO_4 for KH_2PO_4 in the minus K solution, KCl for KH_2PO_4 in the minus P solution, and Na_2SO_4 for $MgSO_4$ in the minus Mg solution. Minor elements were supplied in

the following parts per million of nutrient: boron 0.5, manganese 0.5, zinc 0.05, and copper 0.02. A 0.5 per cent iron tartrate solution was added once weekly at the rate of 1 ml. per liter of nutrient. The growth substances, vitamin B_1 (Merck) and naphthaleneacetic acid, were mixed with the nutrient solution immediately before it was supplied to the plant, each being prepared in aqueous stock solution and used in the dilution of 0.1 mg. per liter of nutrient.

The chrysanthemum plants were harvested on July 10. The roots were separated from the tops at the soil line, and the roots were washed free of adhering sand by dipping them in salt water and then rinsing in distilled water. After being blotted to remove the excess water, they were placed in open paper containers and dried to constant weight in a draft oven. Weights were taken immediately after the plants cooled.

COSMOS AND MARIGOLD

Plants used in this more extensive investigation were chosen because they grow well under the variable conditions of temperature and light which prevail in the Chicago area during the summer months. Both the Harmony marigold and the Burpee Golden cosmos have a comparatively short vegetative period before flowering. And this variety of cosmos has been used in previous experiments of similar nature.

Four-inch glazed pots like those of the previous experiment were used. Quartz sand which had been washed and sifted to free it of organic matter was used. On June 6, selected seeds secured from the Burpee Company of Philadelphia were planted, four or five in each pot at uniform depth. One hundred thirty pots were planted for each of the four treatments of both varieties of plants used, making 1040 pots in all. They were placed at random in rows of ten each on two greenhouse benches.

Germination was almost 100 per cent. Soon after emergence, all but two seedlings were removed from each pot. Within 2 weeks the second seedling was removed, leaving only one in each pot. At this stage, plants of both varieties were very uniform in height and vigor. Complete nutrient solution was supplied twice during the first week after emergence. Beginning on the tenth day, and continuing throughout the course of the experiment, growth substances were added to the nutrient solution and supplied to the plants twice a week. Plants of one group served as controls and received only the complete nutrient solution; plants of another group received naphthaleneacetic acid added to the nutrient solution; a third group received nutrient plus nicotinic acid; and a fourth group received both nicotinic acid and vitamin B_1 in addition to the nutrient solution. All the growth substances were used in the dilution of 0.01 mg. to 1 liter of nutrient. Both marigolds and cosmos were harvested after 8 weeks. The methods used in harvesting and drying were the same as those previously described.

Results and discussion

All chrysanthemum plants of group 1 (table 1), whether or not they were supplied with growth substances in addition to the complete nutrient solution, showed similar vigor. They were very uniform in height and averaged approximately the same number of leaves. The plants which received minus Mg solution showed no detectable variation. All plants, regardless of growth substances, were definitely stunted and showed typical magnesium deficiency. Average dry weight of tops of plants receiving B_1 was slightly greater than that of the controls.

The only group which evidenced any marked response to the addition of growth substances was the one receiving minus K nutrient solution. All plants showed typical potassium deficiency, with browning and final abscission of the leaves. Both the plants receiving B_1 and those receiving naphthaleneacetic acid were more vigorous than the controls, although there was only a slight increase in height. The difference was most apparent in plants which received naphthaleneacetic acid. They showed a significant increase in dry weight. They tended to retain ten or twelve leaves in contrast to an average of nine in the controls, and the leaves were somewhat larger. There was also a tendency for the plants receiving naphthaleneacetic acid to produce one or more stolons. None was formed on the control plants.

The numbers of plants involved in the experiment were too small to justify definite conclusions, but their response gives evidence of the possibility that naphthaleneacetic acid may in some degree compensate for lack of potassium. As previously stated, the experiment with cosmos and marigold constituted the major portion of this investigation. Tables 2 and 3 show little response of the plants to the growth substances. The most striking effect was the marked individual variation in height among the cosmos plants. Each group, regardless of treatment, showed a variation in height from a minimum near 20 cm. to a maximum of over 90 cm. All plants seemed vigorous and healthy, regardless of height. This marked variability illustrates how much an inconstant characteristic might be erroneously attributed to some external factor, such as a growth substance, if plants were observed in small numbers.

Table 2 shows an accompanying variation in dry weight occurring within each group of cosmos plants. A statistical analysis to determine the significance of the variances indicates that the mean differences in weight occurring between each group and the control (table 4) lie well below the mean calculated as significant on the basis of 5 per cent probability. Comparison of the cosmos leaves showed a similar range of size in all groups. No variation in floral initiation among the groups was noted. At the time of harvest, 79 out of 130 control plants were in bud or blossom as compared with 74 in the naphthaleneacetic-acid group, 73 of those receiving nicotinic acid, and 77 receiving both nicotinic acid and B_1.

Growth of the marigolds was much more uniform. Approximately the same number of plants from each group showed floral initiation within 5 weeks. After the terminal flower had formed, axillary shoots developed with noticeable rapidity in all plants. Dry weights (table 5) indicate one noticeable variation: the weight

TABLE 2

BURPEE GOLDEN COSMOS

COMPLETE NUTRIENT (CONTROL)		COMPLETE NUTRIENT WITH					
		NAPHTHALENEACETIC ACID		NICOTINIC ACID		NICOTINIC ACID AND B_1	
TOPS	ROOTS	TOPS	ROOTS	TOPS	ROOTS	TOPS	ROOTS
DRY WEIGHT (GM.) OF RANDOM ROWS OF TEN PLANTS EACH							
37.7	9.8	43.2	11.3	52.0	12.7	55.4	14.4
57.5	13.5	54.3	16.5	53.5	14.3	50.8	13.6
56.9	16.5	53.9	13.5	49.9	12.8	57.3	15.3
52.0	14.2	57.0	16.7	49.2	16.1	59.6	14.7
57.3	16.7	55.5	13.9	58.0	14.8	58.4	14.2
52.8	12.7	53.0	13.5	51.2	14.0	56.5	14.8
56.2	13.4	44.8	12.2	57.3	15.9	46.4	13.3
52.2	14.4	51.6	16.3	56.8	14.1	56.8	15.1
51.8	16.6	49.4	16.7	55.7	14.0	54.4	12.8
50.9	14.4	60.8	14.3	55.6	16.0	55.8	15.0
63.8	15.3	51.8	13.1	62.7	17.0	63.5	17.5
59.0	15.3	52.2	13.5	49.5	13.8	40.0	10.0
39.6	12.2	38.8	11.0	44.6	11.0	50.6	13.0
AVERAGE DRY WEIGHT PER ROW OF TEN PLANTS							
52.9	14.23	51.2	14.04	53.54	14.3	54.3	14.1
AVERAGE DRY WEIGHT PER PLANT							
5.29	1.423	5.12	1.404	5.354	1.43	5.43	1.41

of the total marigold plant, and more definitely the weight of the tops of the plants receiving naphthaleneacetic acid (0.01 mg. per liter of nutrient), have less dry weight than the controls. This variance indicates that the weight lies above the calculated figure of significance at 5 per cent probability. This decreased dry weight illustrates the fact mentioned earlier, that although naphthaleneacetic acid is known to initiate root growth from stems, it may also retard growth as a result of toxic action and thus affect general plant growth.

TABLE 3

HARMONY MARIGOLD

Complete nutrient		Complete nutrient with					
		Naphthaleneacetic acid		Nicotinic acid		Nicotinic acid and B_1	
Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots
Dry weight (gm.) of random rows of ten plants each							
35.6	10.0	32.0	8.6	38.6	10.6	37.0	9.4
31.9	8.6	29.5	9.2	36.6	9.6	38.1	9.7
38.9	8.5	29.9	8.8	3.9	9.3	35.0	9.7
33.7	8.3	31.5	9.1	36.0	8.0	38.0	10.2
32.3	8.6	29.7	9.6	36.9	10.7	36.4	10.8
35.0	10.1	28.1	8.0	36.1	9.7	33.6	11.2
37.0	10.9	32.6	9.8	36.0	10.9	36.0	11.2
39.2	11.8	29.7	9.6	38.0	9.0	38.2	9.5
36.5	11.7	32.9	9.6	38.9	10.9	38.3	10.0
38.5	10.0	37.4	10.7	41.5	12.3	37.8	11.0
40.4	12.4	36.0	11.2	41.2	12.8	42.0	10.2
41.4	13.2	37.1	11.6	43.8	12.6	44.9	13.3
41.3	10.9	35.5	10.5	35.9	9.7	40.2	11.0
Average dry weight per row of ten plants							
37.05	10.4	32.45	9.7	38.3	10.5	38.1	10.6
Average dry weight per plant							
3.705	1.04	3.245	0.97	3.83	1.05	3.81	1.06

TABLE 4

MEAN DRY WEIGHT IN GRAMS OF THIRTEEN ROWS OF COSMOS PLANTS USED AS CONTROLS COMPARED WITH SAME UNDER TREATMENTS INDICATED; ALSO ESTIMATED SIGNIFICANT DIFFERENCES IN WEIGHT BETWEEN CONTROLS AND TREATED PLANTS

	I Control	II Naphthaleneacetic acid	Significant* difference (I and II)	III Nicotinic acid	Significant* difference (I and III)	IV Nicotinic acid and B_1	Significant* difference (I and IV)
Tops	52.9	51.2	5.43	53.5	4.99	54.3	5.44
Roots	14.2	14.0	1.69	14.3	1.47	14.1	1.50
Total plant	67.1	65.2	6.57	67.8	6.12	68.4	6.68

* Significant differences are weights above which, at probabilities of 5 per cent, mean differences may be considered statistically significant.

The dry weights of marigold plants supplied with nicotinic acid, and with nicotinic acid and vitamin B_1 combined, showed no increased growth response. Obviously, although vitamin B_1 and nicotinic acid are necessary for root growth, the supply produced by this plant is adequate for its needs. Consequently the external application of such substances, at least in the concentration used, has neither positive nor negative effect upon total plant growth.

TABLE 5

MEAN DRY WEIGHT IN GRAMS OF THIRTEEN ROWS OF MARIGOLD PLANTS USED AS CONTROLS COMPARED WITH SAME UNDER TREATMENTS INDICATED; ALSO ESTIMATED SIGNIFICANT DIFFERENCES IN WEIGHT BETWEEN CONTROLS AND TREATED PLANTS

	I Control	II Naphthaleneacetic acid	Significant* difference (I and II)	III Nicotinic acid	Significant* difference (I and III)	IV Nicotinic acid and B_1	Significant* difference (I and IV)
Tops	37.0	32.4†	2.58	38.3	2.33	38.1	2.50
Roots	10.4	9.7	1.09	10.5	1.23	10.6	1.09
Total plant	47.4	42.1†	3.48	48.8	3.36	48.7	3.30

* Significant differences are weights above which, at probabilities of 5 per cent, mean differences may be considered statistically significant.

† Weights indicating statistical significance.

Summary

1. Neither vitamin B_1 nor naphthaleneacetic acid supplied to chrysanthemum plants in a concentration of 0.1 mg. per liter of complete nutrient solution produced any apparent growth responses or significant difference in dry weight.

2. Chrysanthemum plants receiving naphthaleneacetic acid in a concentration of 0.1 mg. per liter of minus K nutrient solution showed an increase in dry weight over the controls, indicating that the acid can compensate to some extent for the lack of potassium.

3. No significant difference in dry weight occurred between cosmos plants used as controls and those receiving 0.01 mg. of naphthaleneacetic acid, nicotinic acid, or nicotinic acid and vitamin B_1 per liter of complete nutrient.

4. Marigold plants showed little variation in dry weight when supplied with nicotinic acid, or with a combination of nicotinic acid and vitamin B_1.

5. A significant decrease in total dry weight of the marigold plants supplied with napthaleneacetic acid (0.01 mg. per liter) indicates a retarding effect of the acid on growth.

6. None of the treatments to which the cosmos and marigold plants were subjected resulted in visible effects upon their habit of growth or upon floral initiation.

The writer appreciates the assistance given by members of the department of botany of the University of Chicago during the course of this work.

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PHYSIOLOGICAL AND CHEMICAL RESPONSES OF BEAN AND TOMATO PLANTS TO ALPHA NAPHTHALENE ACETAMIDE AND PHENYLACETIC ACID[1]

CHARLES L. HAMNER[2]

Introduction

The investigations here reported deal with some of the physiological, chemical, and anatomical responses of bean and tomato plants to alpha naphthalene acetamide and phenylacetic acid when applied in nutrient solutions and as emulsion sprays to the tops of growing plants. Records have been made of the gross appearance of the plants, wet and dry weights, and content of total nitrogen, calcium, and phosphorus.

Red Kidney bean and Bonny Best tomato were used as the experimental plants. The general procedure for experiments I–IV was to plant four seeds in a $1\frac{1}{2}$-liter glazed crock containing previously unused white quartz sand. Seven to nine days after planting, when the first primary leaves had expanded, the plants were thinned for uniformity to one and two per pot. The pots were arranged in rows of eight plants each on greenhouse benches. Each entire row was given specific treatment, and the treatments were randomized to permit statistical analyses of the harvested material.

The naphthalene acetamide used in the experiments was first dissolved in 10 cc. of 95 per cent ethyl alcohol and added to the specific quantity of nutrient solution required for each treatment just before watering. The first applications were made when the bean plants were thinned. The plants were harvested, each row separately, shortly after the first flowers appeared.

The concentrations of salts used in making the complete nutrient solution were: 0.0045 mol $MgSO_4$, 0.0045 mol KH_2PO_4, 0.0060 mol $Ca(NO_3)_2$, together with the minor elements. In experiments I, III, and IV lesser amounts of phosphate were used, for example, $\frac{1}{8}$ phosphate means that 0.00025 mol KH_2PO_4 was used instead of 0.0045 mol.

Chemical analyses were made of the plants used in experiment III. The calcium content was determined volumetrically (3). The Kjeldahl-Gunning method was used in determining the total nitrogen content (3). Phosphorus content was determined colorimetrically, using phosphorus molybdate in solution (3).

[1] This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

[2] Agent, Bureau of Plant Industry, U.S. Department of Agriculture.

Experimentation

Alpha naphthalene acetamide supplied in nutrient solution

Experiment I.—Bean seeds were planted May 2, 1941, and thinned to two per pot on May 8. Sixteen different combinations were run. Four concentrations of potassium phosphate in the nutrient solutions were used: complete, $\frac{1}{18}$ and $\frac{1}{36}$ as much phosphate as in the complete nutrient, and no phosphate. In combination with each of these nutrient solutions, a range of four concentrations of naphthalene acetamide—10^{-5}, 10^{-7}, 10^{-9}, and 0—was used.

The plants were given nutrient solution every other day, with or without the addition of the naphthalene acetamide, the total number of applications containing acetamide throughout the experiment being six. The plants were harvested June 3, 1941, and wet and dry weights of tops and roots were measured. As a result of this experiment it was found that 10^{-5} concentration of naphthalene acetamide was the greatest amount that could be used effectively without resultant toxicity to the plants. The most effective range of concentration for increased root development appeared to be between 10^{-6} and 10^{-7}.

Experiment II.—This was performed to determine at what point within this range of naphthalene acetamide concentration (10^{-6} to 10^{-7}) the greatest root development would take place. For this purpose bean seeds were planted June 25 and thinned to one plant per pot on July 1. Eighteen replications of the following five concentrations of naphthalene acetamide were used in complete nutrient solution: 0, 10^{-7}, $10^{-6.67}$, $10^{-6.34}$, and 10^{-6}. Six applications of the solutions containing the naphthalene acetamide were made. On July 25 the plants were harvested and the wet and dry weights of tops, roots, and hypocotyls recorded (table 2).

Experiment III.—Two concentrations of phosphate were used. Nine different treatments were given and six replications of each treatment were run (table 1). The seeds were planted June 20, 1941, and the seedlings selected as in the first two experiments. The plants were harvested July 14. Wet weights of the tops and dry weights of tops, roots, and hypocotyls were recorded. Chemical analyses of total nitrogen, calcium, and phosphorus content were made of the tops, roots, and hypocotyls of the plants that had received treatment numbers 1–6 (tables 1, 3).

Plants grown without phosphorus, experiment I, showed no root stimulation, and those grown on a $\frac{1}{36}$ phosphate level showed only slight root stimulation when supplied with acetamide. All other levels of the phosphate concentration resulted in marked increase in root growth upon application of the acetamide in concentration of 10^{-6}. Top growth was inhibited when the phosphate concentration was $\frac{1}{18}$ or less.

From tables 1 and 2, experiment III, it is obvious that the concentration of naphthalene acetamide in the nutrient solution, and the number of applications, strikingly affect the growth response. One application resulted in a less weight of dry matter of tops of 23.29 gm. as compared with 28.70 gm. Root growth was slightly increased by one application, from 6.89 to 7.24 gm. In the case of root growth, the effects resulting from three applications were similar to those from six. This was not true for top growth.

The data in table 2, experiment II, show that a $10^{-6.67}$ concentration of naphthalene acetamide produced the greatest increase in amount of root growth. Growth of the tops was inhibited with increased concentration of acetamide within the range shown in table 2.

TABLE 1

EXPERIMENT III; BEAN PLANTS

TREATMENT NUMBER	NUMBER OF APPLICATIONS	CONCENTRATION OF POTASSIUM PHOSPHATE (MOLS)	CONCENTRATION OF NAPHTHALENE ACETAMIDE
1	0	0.000250	0
2	6	0.000250	10^{-6}
3	6	0.000250	$10^{-5.5}$
4	0	0.00150	0
5	6	0.00150	10^{-6}
6	6	0.00150	$10^{-5.5}$
7	1	0.00150	10^{-6}
8	2	0.00150	10^{-6}
9	3	0.00150	10^{-6}

The calcium contents of the plants grown in experiment III (table 3) showed a percentage dry weight increase in tops with the acetamide treatment. The greatest percentage increase occurred when the largest amount of acetamide was supplied. A similar trend was noted in the hypocotyls. The roots showed no relative increase in calcium content in the plants of the $\frac{1}{18}$ phosphate group and showed a relative decrease in the $\frac{3}{9}$ phosphate group, the decrease being from 23.34 mg./gm. to 15.63 mg./gm.

The percentage of calcium content of the control tops on $\frac{3}{9}$ phosphate was approximately the same as that of the control roots also on $\frac{3}{9}$ phosphate. The calcium content of the plants on the $\frac{3}{9}$ phosphate nutrient solution that contained $10^{-5.5}$ acetamide showed a greater difference between tops and roots than did those receiving no acetamide, the content being 41.10 mg./gm. for the tops and 15.63 mg./gm. for the roots.

The phosphorus content increased in the tops of the plants treated with concentrations of 10^{-6} and $10^{-5.5}$ naphthalene acetamide. In the $\frac{3}{9}$ phosphate group

TABLE 2

DRY WEIGHT (IN GRAMS) OF TOPS, ROOTS, AND HYPOCOTYLS OF BEAN PLANTS TREATED WITH VARYING CONCENTRATIONS OF ALPHA NAPHTHALENE ACETAMIDE IN SOLUTIONS CONTAINING VARIOUS AMOUNTS OF KH_2PO_4

KH_2PO_4 (MOLS)	CONCENTRATION OF ALPHA NAPHTHALENE ACETAMIDE	DRY WEIGHT (GM.)		
		TOPS	ROOTS	HYPOCOTYLS
EXPERIMENT I				
0	0	7.91	4.74	.....
0	10^{-9}	7.98	4.64	.....
0	10^{-7}	7.28	4.54	.....
0	10^{-5}	2.01	1.97	.....
0.000125	0	13.20	6.24	.....
0.000125	10^{-9}	14.31	7.01	.....
0.000125	10^{-7}	12.63	6.54	.....
0.000125	10^{-5}	3.13	3.47	.....
0.000250	0	20.30	7.90	.....
0.000250	10^{-9}	17.91	7.90	.....
0.000250	10^{-7}	15.58	8.87	.....
0.000250	10^{-5}	3.50	3.74	.....
0.0045	0	28.55	7.90	.....
0.0045	10^{-9}	28.67	7.80	.....
0.0045	10^{-7}	26.30	8.70	.....
0.0045	10^{-5}	3.93	3.67	.....
EXPERIMENT II				
0.0045	0	28.3	6.52	2.07
0.0045	10^{-7}	25.7	7.77	1.87
0.0045	$10^{-6.67}$	22.2	8.90	1.63
0.0045	$10^{-6.34}$	19.1	8.63	1.50
0.0045	10^{-6}	15.9	7.56	1.20
EXPERIMENT III				
0.00025	0	22.68	7.11	2.43
0.00025	10^{-6}	16.13	8.91	1.75
0.00025	$10^{-5.5}$	8.70	8.28	1.78
0.0015	0	28.70	6.89	2.12
0.0015	10^{-6}	17.83	8.57	1.53
0.0015	$10^{-5.5}$	7.78	7.65	1.37
0.0015	10^{-6}*	23.29	7.24	2.05
0.0015	10^{-6}†	20.90	7.70	2.16
0.0015	10^{-6}‡	21.70	8.34	2.08

* One application. † Two applications. ‡ Three applications.

the control tops showed a phosphorus content of 3.84 mg./gm. In the same phosphate group the tops of the plants given the acetamide in concentration of 10^{-6} contained 4.19 mg./gm. phosphorus, while those treated with a $10^{-5.5}$ solution contained 6.40 mg./gm. The plants that had received the $\frac{3}{9}$ phosphate nutrient solution showed a much higher phosphorus content in the tops and roots than those that had received the $\frac{1}{18}$ phosphate solution. The difference between the phosphorus contents of the tops and of the roots increased with increasing amounts of the acetamide supplied.

TABLE 3

EXPERIMENT III; BEAN PLANTS

CALCIUM, PHOSPHORUS, AND TOTAL NITROGEN CONTENT OF PLANTS TREATED WITH AND WITHOUT ALPHA NAPHTHALENE ACETAMIDE IN NUTRIENT SOLUTION

WEIGHT IN MG. PER GM.

SUBSTANCE DETERMINED	PLANT PART	CONCENTRATION OF ALPHA NAPHTHALENE ACETAMIDE IN NUTRIENT SOLUTION					
		0.00025 MOL KH_2PO_4			0.0015 MOL KH_2PO_4		
		0	10^{-6}	$10^{-5.5}$	0	10^{-6}	$10^{-5.5}$
Ca	Tops	23.37	28.60	38.94	23.67	29.98	41.10
	Roots	14.21	14.29	13.03	23.34	17.16	15.63
	Hypocotyls	9.09	11.57	11.97	8.58	10.67	12.57
P	Tops	1.80	2.11	2.40	3.84	4.19	6.40
	Roots	0.84	0.89	0.94	2.20	2.15	2.20
	Hypocotyls	0.93	1.08	1.48	2.22	2.58	3.07
Total nitrogen	Tops	39.66	42.73	44.50	38.96	40.44	40.18
	Roots	30.79	35.88	35.90	28.67	33.47	38.01
	Hypocotyls	16.55	17.78	20.85	14.83	15.43	23.60

The total nitrogen content was greater in the tops, roots, and hypocotyls of all plants treated with acetamide. In the $\frac{1}{18}$ phosphate group the total nitrogen content for the tops showed a progressive percentage increase with increase in concentration of acetamide. The roots of the plants that had received acetamide in concentrations of 10^{-6} showed a marked percentage increase in nitrogen content over the controls, whereas those that had received $10^{-5.5}$ acetamide showed only a slight increase over those having received 10^{-6}. In the $\frac{3}{9}$ phosphate group there was a progressive percentage increase in total nitrogen in the roots with increasing concentration of acetamide. The tops in this group showed an increase with acetamide treatment but displayed no great differences due to the different concentrations. The hypocotyls in both groups showed marked percentage increase with increasing acetamide concentration.

Histological sections of the roots of bean plants showed the following differences between the plants treated with a concentration of naphthalene acetamide of 10^{-6} and the controls. The number of secondary roots of the treated plants was greater than those of the untreated, although many of these roots were short and stubby. The diameter of the younger roots of the treated plants was approximately twice as great as that of the controls. The cells of the embryonic region of the treated roots showed much denser cytoplasm. The subepidermal cortical cells showed many more radial divisions of the cells as contrasted with the tangential divisions in the controls. A number of the cortical cells were enlarged and spherical, resulting in many air spaces. The most striking feature of the treated plants was the very early maturation of the elements of the xylem and phloem. Maturation in most of the roots took place within less than 0.6 mm. of the apical meristem. Division of the cells of the pericycle over the xylem points was less pronounced in the controls than in the treated plants. In the treated plants pericyclic activity was most pronounced back from the apical meristem, where the xylem was nearly mature. At this level there was a bulbous enlargement of the root. In a few of the treated roots radial division of the epidermal cells occurred. In some of the roots of the treated plants no differences from the controls could be observed.

Alpha naphthalene acetamide applied as emulsion spray to the leaves

Experiment IV.—On April 29, 1941, four seeds of bean were planted in each of 1200 glazed crocks in clean unused quartz sand. Immediately after planting, the pots were divided into three lots, one lot receiving complete nutrient solution, another a similar solution containing only $\frac{3}{9}$ as much phosphorus, and the third lot containing $\frac{1}{18}$ as much phosphorus. The pots were then completely randomized in rows on four greenhouse benches, there being twenty-one replications of each treatment. On May 6 the number of plants per pot was reduced to one. On May 7 the primary leaves had completely unfolded but were not of full size. The epicotyl had not yet begun to elongate. On the afternoon of this day, half the plants of each lot were sprayed with an emulsion of naphthalene acetamide in the amount of 400 mg./l. They were given a similar spraying on May 15 and were watered with the respective nutrient solutions on May 14, 19, and 22, and with distilled water on May 17.

All the plants grew well. The tops of those sprayed with the acetamide emulsion were not so large as those left unsprayed. Very little difference in growth was noted between the plants supplied with the different quantities of phosphorus in the nutrient medium. On May 30 the tops, and on June 2 the roots, were harvested. Each individual row was kept separate and the dry weights determined. A summary of the results is given in table 4.

PHENYLACETIC ACID SUPPLIED IN NUTRIENT SOLUTION TO BEAN AND TOMATO PLANTS

EXPERIMENT V.—On April 14, four seeds of bean were planted in each of a number of glazed crocks containing unused white quartz sand and then sprinkled

TABLE 4

EXPERIMENT IV; BEAN PLANTS

	Complete				$\frac{3}{9}$ Phosphorus				$\frac{1}{18}$ Phosphorus			
	Tops		Roots		Tops		Roots		Tops		Roots	
	Control	Sprayed	Control	Sprayed	Control	Sprayed	Control	Sprayed	Control	Sprayed	Control	Sprayed
	Dry weights of tops and roots per 8 plants (in grams)											
Weight	24.4	22.3	10.1	10.1	25.2	22.9	11.1	11.6	24.4	22.2	10.3	10.4
	Calcium and phosphorus content of top leaves and stems (in mg./gm.)											
Ca	12.3	12.4	8.2	8.3	15.4	14.1	13.3	11.4	17.9	16.6	14.8	11.2
P	5.5	6.4	3.3	3.3	4.8	5.0	3.4	3.4	2.4	2.5	1.4	1.4

TABLE 5

EFFECT OF PHENYLACETIC ACID SUPPLIED IN NUTRIENT SOLUTION UPON TOPS AND ROOTS OF BEAN PLANTS, EXPRESSED IN GRAMS PER 10 LOTS OF PLANTS

Concentration	Tops	Roots
10^{-6}	36.8	14.3
10^{-7}	36.7	14.3
10^{-8}	37.4	14.8
10^{-9}	37.0	15.2
10^{-10}	36.8	14.7
10^{-11}	37.3	14.6
10^{-12}	37.8	16.0
Control	39.2	15.8

with tap water. On April 21 the plants were thinned to two per crock and divided into ten lots of eighty crocks each. These lots were arranged in sequence on four greenhouse benches. Each lot was subdivided into eight rows of ten plants each. The rows in each lot were randomized by drawing the numbers according to chance. Within each lot one row served as a control; the others received phenyl-

acetic acid in complete nutrient solution as follows: 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10}, 10^{-11}, 10^{-12}. Waterings with the respective solutions were made on April 22, 25, 29, and May 2, 6, and 9. All were given one watering with distilled water on May

TABLE 6

EXPERIMENT VI; TOMATO PLANTS

DRY WEIGHT OF TOPS AND ROOTS (IN GRAMS) ADDED TO OBTAIN DRY WEIGHT OF TOTAL PLANT. STATISTICAL ANALYSIS MADE ON BASIS OF DRY WEIGHT OF TOTAL PLANT; JULY, 1941

Row	Concentration of phenylacetic acid in nutrient solution							
	Control	10^{-13}	10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}
	Tops							
1	23.1	23.2	25.2	25.3	23.6	25.0	24.7	26.2
2	21.6	23.7	26.2	24.6	24.5	25.2	24.8	24.7
3	23.8	25.3	26.3	25.3	24.6	26.7	26.6	27.2
4	22.9	26.2	24.4	26.7	24.4	25.2	24.9	22.4
5	22.7	26.9	24.5	25.4	24.1	26.3	27.3	25.4
6	20.3	26.0	23.6	26.0	26.8	27.6	25.5	24.6
7	22.3	23.8	25.6	22.6	24.2	22.6	22.9	22.7
8	22.2	24.1	23.3	24.0	22.4	23.7	24.9	24.0
9	19.4	23.5	23.6	28.1	23.7	26.2	21.7	24.8
10	20.3	26.1	25.0	25.0	22.6	20.9	29.4	22.4
11	20.4	23.6	22.5	22.1	24.2	22.0	22.8	23.5
12	21.1	23.0	23.0	23.8	26.4	24.8	22.7	24.8
Sum	260.1	295.4	293.2	298.9	291.5	296.2	298.2	292.7
Average	21.7	24.6	24.4	24.9	24.3	24.7	24.9	24.4
	Roots							
1	12.1	11.5	9.1	11.2	10.5	11.2	10.4	10.8
2	10.7	11.5	10.5	10.8	15.3	11.3	9.3	9.1
3	13.8	12.0	11.3	11.3	10.5	11.6	11.7	10.3
4	11.2	12.7	9.2	10.8	10.9	14.3	11.2	13.8
5	10.0	12.1	13.8	11.6	11.8	11.1	12.5	10.4
6	11.2	13.0	10.4	9.6	13.6	12.4	11.3	10.4
7	9.6	9.2	12.5	10.1	11.1	8.7	8.8	8.7
8	10.4	9.2	9.8	11.7	9.4	11.3	12.7	10.7
9	8.4	9.4	8.2	13.1	9.4	10.8	8.4	9.3
10	11.1	13.0	12.1	11.8	10.5	12.5	15.6	9.6
11	8.7	9.8	12.0	13.1	10.8	10.1	10.4	9.2
12	10.0	8.5	9.5	9.8	11.7	10.2	10.7	11.2
Sum	127.2	131.9	128.4	134.9	135.5	135.5	133.0	123.5
Average	10.6	11.0	10.7	11.2	11.3	11.3	11.1	10.3

11. The tops and roots of each row were harvested separately on May 12, and their dry weights were determined. Table 5 shows a summary of results, analysis of which shows no significant differences owing to the treatments.

EXPERIMENTS VI, VII, VIII.—Tomato plants were used in three experiments in which phenylacetic acid was used in the nutrient solution. Experiments VI and VII were conducted at Beltsville, Maryland, and experiment VIII at Chicago, Illinois. Tomato seeds were planted in quartz sand and watered with the complete nutrient solution as previously described. The first application containing phenylacetic acid was made when the plants were 3–4 inches in height, and subsequently two or three times during each succeeding week. The phenylacetic acid was first dissolved in ethyl alcohol and then added to the nutrient solution. The concentration ranged from 1 part in 10,000 to 1 part in 1,000,000,000,000. Nine

TABLE 7

DRY WEIGHT OF TOPS AND ROOTS OF TOMATO PLANTS UNDER VARIOUS TREATMENTS OF EXPERIMENTS VI, VII, AND VIII, EXPRESSED IN GRAMS DRY WEIGHT PER 8 PLANTS

CONCENTRATION OF PHENYLACETIC ACID	EXPERIMENT VI		EXPERIMENT VII		EXPERIMENT VIII	
	TOPS	ROOTS	TOPS	ROOTS	TOPS	ROOTS
10^{-4}	.....	.....	.....	.....	43.5	11.0
10^{-5}	24.4	10.3	.....	.....	48.2	12.0
10^{-6}	24.9	11.1	97.9	29.0	50.4	12.1
10^{-7}	24.7	11.3	.....	.....	51.1	12.2
10^{-8}	24.3	11.3	92.8	27.5	53.5	12.05
10^{-9}	24.9	11.2	.....	.....	51.0	12.1
10^{-10}	24.4	10.7	.....	.....	.....	.....
10^{-11}	24.6	11.0	.....	.....	.....	.....
10^{-12}	.....	.....	94.5	29.5	.....	.....
0	21.7	10.6	95.0	27.8	51.5	12.1
0*	.....	.....	96.1	27.1	.....	.....

* Plus alcohol.

to twelve replications of each treatment were made, using eight crocks as a unit. The plants were harvested at the time of their first blooming. Statistical analyses were made of the total dry weight of the plants.

For experiment VI, tomato plants 3–4 inches high received their first treatment on June 30. Eight different treatments were used, and twelve replications of each were made. The concentration of the phenylacetic acid ranged from 10^{-6} to 10^{-12}. A control to which no acid was added was used for comparison. A 200-cc. solution was added to each pot containing one plant, two or three times a week. The plants were harvested when the first blossom cluster opened, on July 30. All plants receiving treatments with the acid showed approximately a 15 per cent increase in top growth over that of the controls and approximately a 7 per cent increase in root growth. The results are given in table 6, which is representative of the data taken in all three experiments.

Experiment VII, also run at Beltsville, was conducted to determine whether or not greater dilutions of phenylacetic acid would result in increased growth. More favorable weather conditions prevailed during the course of this experiment than in the case of experiment VI, so that the plants grew larger. The tomato plants received their first treatment August 16. The acid was applied in concentrations of 10^{-6}, 10^{-8}, and 10^{-12}. Two controls were used, one containing nutrient solution alone, the other containing nutrient solution plus the amount of alcohol used as a carrier for the acid. The plants were harvested when the third blossom cluster opened, on September 30. Dry weights of tops and roots were determined. No significant differences between the plants receiving the various treatments were obtained in this experiment (table 7).

Experiment VIII, carried on in Chicago, ran concurrently with experiment VI. The concentration range of phenylacetic acid used was from 10^{-4} to 10^{-9}. The plants received their first application August 14. Three treatments per week were administered. The plants were harvested September 23. The stronger concentrations, 10^{-4} and 10^{-5}, produced a decrease in both top and root growth. A slight increase in top growth (approximately 5 per cent) was obtained as a result of treatments with the acid in the concentration of 10^{-9}.

Discussion

MITCHELL and STEWART (**5**) found that when alpha naphthalene acetamide was applied in high concentrations as an emulsion spray to the tops of bean plants, increased root growth and decreased top growth resulted. When applied in low concentrations, top growth was increased. When applied in lanolin paste to decapitated bean plants, mobilization of solid materials toward the treated region resulted. KRAUS and MITCHELL (**1**), in a later experiment, found that spraying the tops with an emulsion of alpha naphthalene acetamide also resulted in increase of the amount of cell-wall thickening and development of secondary xylem in the stem and petioles.

MITCHELL (**4**) reported that when a lanolin mixture of alpha naphthalene acetamide was applied to the stem of bean plants the percentage of starch, dextrin, and sugar in the roots, hypocotyls, and first internode was greater than in control plants. He also reported an inhibition of the translocation of carbohydrates from the leaves. LAUDE (**2**) reported increased root growth of bean when low concentrations of naphthalene acetamide were supplied in the nutrient solution.

In the present investigation, when naphthalene acetamide was supplied in the nutrient solution, the plants reacted in a manner somewhat similar to those to which it had been supplied as a spray or in a lanolin paste. Leaf expansion was inhibited and curling and thickening of the leaf blade was noticeable. Relatively less growth of the tops was one of the most striking reactions. Root growth was

greater than that of the controls by as much as 37 per cent in some cases. The percentage of calcium, phosphorus, and nitrogen in the tops was greatly increased, although the absolute amount was less because of the less amount of total growth made by the treated plants.

The increased root growth might be explained by the diversion of carbohydrates to the roots, which may in part account for the relative percentage increase of calcium, phosphorus, and nitrogen and their accumulation in the tops. The increased ash content of the tops of the treated plants is correlated with decreased top growth. The greater root growth was accompanied either by no change in calcium and phosphorus of the root or by a percentage decrease in both of them. Thus the percentage of calcium and phosphorus in the plant tissue is apparently negatively correlated with the total dry weight.

In the control plants grown with an adequate supply of all the minerals, the percentages of calcium in the tops and in the roots were approximately the same, while in some of the plants treated with the acetamide the calcium content was two and one-half times as great in the tops as in the roots. Compared with their respective controls, there was a much wider difference between the calcium and phosphorus contents of the tops than of the roots on the basis of increased content of acetamide in the nutrient solution. The ratio of the calcium of the tops to that of the roots of the untreated plants was 1:1, while that of the treated plants was 2.5:1. The ratio of top to root growth of the control plants was 4:1, while that of the treated plants was 1:1. There would appear to be an inverse relationship between the top-root ratio of dry matter and the top-root ratio of calcium content.

As reported by MITCHELL and STEWART (5), increased top growth resulted from spraying the tops of bean plants with dilute emulsions of naphthalene acetamide; in the experiments reported here in no instance was an increase obtained when this substance was supplied in the nutrient solution. Greatest increase of root growth resulted when a concentration of 0.34 mg./l. of naphthalene acetamide was supplied in the nutrient solution; increase of root growth, as reported by MITCHELL and STEWART, resulted when 625 mg./l. was used as an emulsion spray.

When alpha naphthalene acetamide was applied to the tops in the form of a spray in a manner similar to that reported by MITCHELL and STEWART (except that the plants were allowed to grow much longer after the initial treatment), the plants showed a slight depression of top growth and dry weight and no differences in dry weight of the roots. No increase was found in the content of the uppermost leaves or stems owing to the spray applied. There was, however, greater content of calcium in the tops in those cultures where there was less phosphorus in the nutrient solution; but this difference was independent of any of the spraying treatments.

Summary

1. Alpha naphthalene acetamide when applied in the nutrient solution results in less top growth and increased root growth of Red Kidney bean plant.

2. Relative percentage contents of calcium, phosphorus, and nitrogen in the bean plant are increased by the presence of naphthalene acetamide in the nutrient solution.

3. The xylem and phloem mature earlier and are more extensive in the roots of bean plants treated with naphthalene acetamide in the nutrient solution.

4. Spraying the tops of bean plants with naphthalene acetamide did not result in increase of calcium or phosphorus content in the top leaves or stems, or result in increased dry weight of the plants.

5. Phenylacetic acid when supplied in the nutrient solution to bean plants did not significantly increase the total dry weight.

6. Phenylacetic acid when supplied in the nutrient solution to Bonny Best tomato may increase top and root growth in young plants. The evidence, however, is not conclusive.

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POLARITY OF AUXIN TRANSPORT IN INVERTED TAGETES CUTTINGS[1]

F. W. WENT

(WITH ONE FIGURE)

Ever since the early work on regeneration of roots and outgrowth of buds on cuttings, the polarity of this phenomenon has been stressed. Notable exceptions to this behavior were found only in branches with preformed root primordia. More recently it was shown that a reversal in the polarity of root formation could also be achieved by applying high concentrations of auxins or auxin-like substances. Then roots appear at the place of application regardless of the original polarity of the plant.

This polarity in organ formation was demonstrably due to polar auxin transport within the branch. It had not been possible to reverse the polarity of auxin transport in the living cell (1), and the apparent lack of polarity in certain experiments could be attributed to auxin leakage through nonliving tissues (3) or along wet surfaces (7). For these reasons it seemed of interest to study the polarity of auxin transport in stem pieces which were functioning in a polarly inverted direction.

The question was: If a piece of stem has produced shoots near its original base which are supplied with water and salts through roots which developed near its original apex, in which direction will the auxin then move through the stem? For it is clear that in this case water, salt, and organic food transport are inverted.

To produce such stem pieces with inverted food transport, cuttings of *Tagetes* were made so as to include 2–3 nodes. They were placed either with their base or with their apex in auxin solution and then planted in sand. Figure 1 shows the position of the cuttings. In this figure and throughout the following discussion, apex and base will be used to designate the original or morphological apex and base of the stem piece, irrespective of its position toward gravity. In both cases roots developed at the treated region of the cutting. The axillary buds on the middle nodes were removed, and thus in the upright cuttings only the buds near the apex grew out, and in the inverted cuttings only those at the original base grew out. This left a portion of stem approximately 10 cm. long between roots and growing lateral shoots, which was ample for auxin transport determination.

The first inverted cuttings tested were kindly supplied from the greenhouses at Beltsville by Dr. E. J. KRAUS. Although the cuttings were not in the best condi-

[1] Report of work undertaken as part of a cooperative project between the California Institute of Technology and the Bureau of Plant Industry, U.S.D.A.

tion upon arrival in Pasadena, the following results with auxin transport were obtained. Stem sections 6–8 mm. long were cut from the inverted stems (fig. 1, *2*) and from the normal lateral shoots (fig. 1, *3*). They were each placed on a 1-mm. thick agar block 5 × 5 mm. An agar block of the same dimensions containing 10 mg./l. or 50 mg./l. indoleacetic acid was placed on top of the section, and 2 hours later the bottom blocks were analyzed (after cutting them into four pieces)

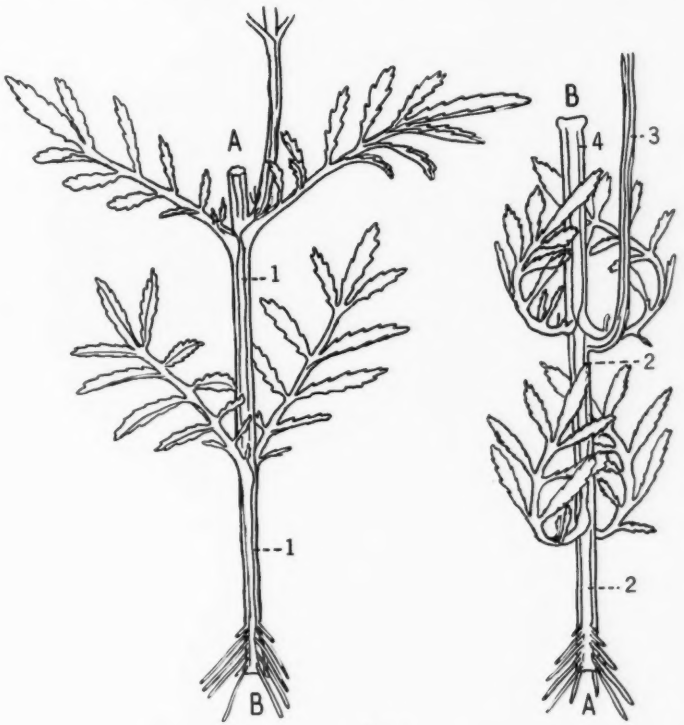

FIG. 1

on four standard *Avena* test plants each. In eight stem pieces from lateral shoots in which normal apex-to-base auxin transport was tested, three clearly showed transport. In eight pieces with inverse transport all tests were negative. It can be concluded that the lateral shoots on these inverted cuttings have normal polar auxin transport. In nine sections from the inverted part of the cutting, in which auxin moved from original apex to base, five gave transport out of nine. Of nine sections with auxin applied at the base, seven gave strong transport. Thus the polarity in auxin transport had disappeared in the inverted stem portion of the cutting.

In a subsequent set of tomato and *Tagetes* cuttings, made in winter, the original polarity was still much in evidence in the inverted cuttings, and only a few indications of inverted auxin transport were found.

A conclusive set of auxin transport determinations was carried out on *Tagetes* cuttings made July 25, 1939. All cuttings had two nodes and were treated, either at the base or the apex, with 100 mg./l. indoleacetic acid for 18 hours. They were potted on August 2, when all had formed large numbers of roots at the treated end. In table 1 all auxin transport determinations with these cuttings have been compiled. In most cases two rows of twelve test plants each were used to determine the amount of auxin transported through the 6–8 mm. stem sections. The values of both test rows are included in the table. It will be seen that there is considerable variability in the auxin transport, but that qualitatively the figures leave no room for doubt. In the main stem of normal upright cuttings auxin moves readily from apex to base, but (except in one case, presumably an experimental error due to inversion of the stem section) no auxin is transported from base toward the apex. In the inverted cuttings the inverted stem between roots and insertion of the lateral branch (fig. 1, *2*) has a different behavior. Immediately after inverting the cuttings the polarity was still normal. After lateral shoots were well developed, 21 days after inversion, this original polarity was still very pronounced, but small amounts of auxin seem to move already in base-to-apex direction.

Two, 3, and 6 weeks later, a strong auxin transport, almost as great as in the apex-to-base direction, was found in the inverse direction. There is no doubt about this effect, which agrees with that found in the preliminary determinations. It is interesting that the polarity as such is not reversed, but that the auxin transport in the original direction continues at about the same rate and that a second auxin transport in the opposite direction is added. This is brought about by the inverse position of roots and shoots in regard to the old stem, and it is not a function of the effect of gravity. This is indicated by the auxin transport through the inverted stem portion basal from the lateral shoots (*4* in fig. 1 and table 1). With both roots and shoots inserted apically no inverse auxin transport developed, nor was there any disturbance of polarity in the lateral shoots of inverted cuttings.

This physiological polarity is in perfect agreement with previous views, especially by VÖCHTING, concerning morphological polarity of plant organs and tissues. VÖCHTING (**4**) had concluded from anatomical evidence that every living cell has a polar structure, and that the morphological and physiological polarity of organs is due to the polarity of their cells. Especially convincing was the evidence in his experiments with inversely implanted pieces of *Beta vulgaris* roots. Although in many cases junction of the tissues of host and graft occurred, irregular overgrowth indicated that they differed from normally implanted grafts. Anatomical investigation showed that where two apical or two basal ends of tissues were in

juxtaposition, no direct vascular connections between them were established. But always the cells growing out from both apical or both basal ends seemed to repulse each other, bending around in a half circle, so that ultimately when the cells from graft and host joined they had the correct polar position of base adjoining apex. A polar reversal of the implanted tissue was never observed, nor a base-to-base junction of cells, so that the polarity of the cell was also absolute. Later VÖCHTING (5) drew the same conclusions from grafting and regeneration in *Brassica oleracea*

TABLE 1

AMOUNT OF AUXIN (IN DEGREES STANDARD AVENA TEST CURVATURES) COLLECTED AT ONE END OF STEM SECTIONS OF TAGETES CUTTINGS 6–8 MM. LONG, WHEN 50 MG./L. INDOLEACETIC ACID IS APPLIED AT OTHER END. EACH VALUE IS MEAN OF AT LEAST 12 TEST PLANTS. NUMBERS IN BRACKETS REFER TO STEM PORTIONS OF FIGURE 1 (A–B, APEX TO BASE TRANSPORT; B–A, BASE TO APEX TRANSPORT)

DAYS BETWEEN MAKING CUTTINGS AND AUXIN TRANSPORT TEST	NORMAL UPRIGHT CUTTINGS (1)						INVERSE CUTTINGS (2)						LATERAL SHOOTS ON INVERSE CUTTINGS (3)		INVERSE CUTTING: PORTION OF MAIN STEM BASAL OF LATERAL SHOOTS (4)	
	APEX TO BASE TRANSPORT			BASE TO APEX TRANSPORT			APEX TO BASE TRANSPORT			BASE TO APEX TRANSPORT						
	A–B		MEAN	B–A		MEAN	A–B		MEAN	B–A		MEAN	A–B	B–A	A–B	B–A
15	....	....	12.4	....	.....	0.5	....	....	.....	....	....	.....	....	....	....	....
21	17.6	14.2	15.9	0.7	0.3	0.5	11.6	7.9	9.7	1.1	2.7	1.9	....	....	....	....
36	13.7	11.7	12.7	0.7	1.1	0.9	12.1	16.6	14.3	7.7	11.6	9.6	....	....	....	....
43	13.8	19.9	16.8	0.6	(11.0)	0.6	16.7	12.5	14.6	5.8	11.4	8.6	....	....	....	....
66	15.2	18.1	16.6	0.2	0.3	0.2	31.4	27.0	29.2	6.0	18.3	12.1	21.5	0.5	26.0	0.5
Mean			14.9			0.5			16.9			8.0	21.5	0.5	26.0	0.5

tubers. Development of inverted cuttings of *Salix* (6), rooted at their apex, confirmed the earlier results. In the beginning, growth of these cuttings was slow, but in many the original inhibition was overcome in the course of years. Then a large overgrowth had developed on such a cutting. The polarity of the cells in this overgrowth was irregular, but finally—where this overgrowth touched the thickened adventitious root—a continuous series of cells with base-to-apex junctions joined shoot with root. Thus in addition to the inverted tissues of the original stem, new tissues with the opposite polarity had developed. In an extensive anatomical study, NEEFF (2) also found that with inversion of function in stems and roots a reorientation of cells occurs, which finds its origin in form changes of the cambial cells. They bend around, and the new vascular tissue derived from these reversed cambial cells has also an inverted morphological polarity.

These cases give a probable explanation of the behavior of polar auxin transport in inverted *Tagetes* cuttings. The mature cells of the stem all retain their original polarity; therefore auxin transport in the basal direction remains unchanged. In line with VÖCHTING's and NEEFF's results, it has to be assumed that the outgrowth of buds basally from the roots results in the laying down of new tissues or perhaps new vascular bundles. In these new vascular elements, connecting root and shoot, the cells have the opposite polarity for auxin transport. As a result the gradual development of an auxin transport from original base to apex would be expected, not interfering with the existing polar mechanism and starting some time after rooting of the inverted cutting. It is likely that such a new vascular system with opposite physiological polarity could be observed anatomically. This still remains to be done. With this explanation as a good possibility, there is no certain basis as yet for assuming that the polarity of auxin transport in a cell can be inverted, even though the organ in which the cell occurs may be functioning in an inverted position.

Summary

In inverted *Tagetes* cuttings, which have developed roots at their original apex and shoots near their base, the original polar auxin transport persists, but in addition a new auxin transport from the original base toward the apex develops. The first indication of this inverse transport occurs 3 weeks after starting the inverted cutting, and after a month both normal and inverse auxin transports occur simultaneously in the inversely functioning portion of the stem.

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SYNTHETICALLY PRODUCED SUBSTANCE B

CLAIR L. WORLEY

Introduction

NIELSEN and HARTELIUS (2) succeeded in forming substance B by chemical means. Their first results were obtained by autoclaving the "*Rhizopus*-nutrient-solution" in the presence of filter paper and assaying by dry weight yields of *Aspergillus niger*. They also showed that the mineral salts played no part, while the important factors were the nitrogen source, the carbon source, and the filter paper. Various nitrogen sources were tried, and the results indicated that all organic ammonium salts were effective, that NH_4Cl had no effect, and that $NaNO_3$ produced an inhibiting factor. Tests with various carbon sources showed that all were more or less effective. Six different filter papers were tried, and Schleicher and Schull no. 597 proved to be the most effective. They also state that the ash of the filter paper gave results similar to those of the filter paper itself. This synthesized substance not only functioned similarly to substance B of the *Rhizopus* filtrate, but also—like the latter—it was soluble in water, insoluble in ether, and stable to oxidation by perhydrol.

In a subsequent paper (3), these workers demonstrated the production of this active substance by autoclaving 2 per cent lactic acid and 2 per cent glucose, at 135° C., with filter paper or products obtainable from it. These last in decreasing order of effectiveness are: filter paper, ash of filter paper, HCl-soluble substances. NIELSEN and HARTELIUS also produced this active factor by autoclaving glucose and ammonium tartrate in the presence of $ZnSO_4$. Their ultimate conclusions were: (*a*) filter paper has little or no effect on the growth of *Asp. niger*, but serves with substance B as a co-factor; (*b*) substance B can be formed chemically by autoclaving a nutrient carbon source and an ammonium salt in the presence of $ZnSO_4$, filter paper, or various fractions of the latter.

DAGYS (1) obtained similar results. He showed that autoclaving malic acid, or one of its salts, and fructose in the presence of filter paper resulted in the formation of a substance (substance B?) effective with *Asp. niger*. He likewise showed that the ash of birch sap would adequately substitute for the co-factor of the filter paper. He, like NIELSEN and HARTELIUS, demonstrated that the physico-chemical properties of the synthetic substance B were similar to the one produced metabolically by *Rhiz. suinus*.

Several series of experiments were arranged to retest the results of the investigators just referred to, at the same time using another organism, *Rhiz. suinus*, and

with the hope of obtaining a better insight into the processes taking place. Any methods or procedures not outlined in detail below have been so treated in a previous section of this report (4). In several of the following experimental tests filter paper and a nutrient solution are required. Unless stated to the contrary, the filter paper used was Reeve Angel no. 202, circles 25 cm. in diameter. The following stock solutions were prepared and used in these tests.

NH_4-tartrate (40.912 gm. per liter)	25 ml.
$MgSO_4$-$7H_2O$ (9.860 gm. per liter)	5 ml.
KH_2PO_4 (10.900 gm. per liter)	5 ml.
Dextrose (66.700 gm. per liter)	15 ml.
Ferric tartrate (0.1 saturated solution)	3 drops
H_2O (distilled)	50 ml.
Total	100 ml.

The data presented in tables 1–3 represent several replicas; enough to assure a biological significance of the results (that is, the factor F represents the mean of nine original values).

Experimentation

I. Effect of varying quantity of filter paper used

In this test the filter paper quantity, which is specified in each of the following solutions, was separately shredded, suspended in water, autoclaved for 20 minutes at 15 pounds' pressure, cooled to room temperature, and filtered. These solutions are:

C = control (distilled H_2O only)
1 part = $\frac{1}{8}$ piece filter paper in 50 ml. H_2O
2 parts = $\frac{1}{4}$ piece filter paper in 100 ml. H_2O
4 parts = $\frac{1}{2}$ piece filter paper in 200 ml. H_2O
8 parts = 1 piece filter paper in 400 ml. H_2O
16 parts = 2 pieces filter paper in 800 ml. H_2O
32 parts = 4 pieces filter paper in 1600 ml. H_2O

Each of these aqueous solutions was filtered and reduced to 50 ml. by partial evaporation, prior to testing. This was then combined with the other stock solutions (with 25 ml. NH_4-tartrate solution, 5 ml. $MgSO_4$-$7H_2O$ solution, etc.). This procedure yielded 100 ml. of each test solution. These were portioned into 25-ml. aliquots and placed in 125-ml. Erlenmeyer flasks, sterilized by autoclaving, inoculated with the *Rhizopus* spores, and cultured under the standardized conditions.

The results in table 1 confirm those obtained by Nielsen and Hartelius (2, 3) and by Dagys (1), suggesting that filter paper contains a co-factor for certain

microörganisms. Although the extent of growth was related to the quantity of filter paper used, the increases were less than those previously reported (3). With this confirmation of the beneficial effect of filter paper, an effort was made to investigate the specific agencies involved.

TABLE 1

EFFECT OF INCREASED CONCENTRATIONS OF CO-FACTOR IN FILTER PAPER ON GROWTH OF RHIZ. SUINUS

CULTURE	AVERAGE DRY WEIGHT OF RHIZOPUS MYCELIUM (MG).	F±SE	SPORULATION
Control	81.6	1.00	++
1 part	83.2	1.02±0.009	+++
2 parts	89.6	1.10±0.015	+++
4 parts	95.0	1.16±0.009	+++
8 parts	96.7	1.18±0.012	+++
16 parts	102.8	1.26±0.025	+++
32 parts	104.3	1.32±0.011	+++

II. EFFECT OF FILTER PAPER WHEN TREATED VARIOUSLY

In this series of experiments each of the following reagents was autoclaved with 50 ml. of water and $\frac{1}{8}$ sheet of filter paper. The paper was removed by filtration, and the filtrate, after cooling, was brought to mark. To this filtrate was added the remaining stock solutions, as in test I. The substances constituting the solutions of the original autoclavings are:

C. No filter paper (control).
I. Filter paper+all stock solutions.
II. Filter paper+water.
III. Filter paper+NH_4-tartrate solution.
IV. Filter paper+$MgSO_4$ solution.
V. Filter paper+KH_2PO_4 solution.
VI. Filter paper+dextrose solution.
VII. Filter paper+ferric tartrate solution.
VIII. Filter paper ashed in muffle furnace; ash added to nutrient solution, prepared by combination of stock solutions; mixture agitated for 30 minutes, then filtered and autoclaved.
IX. Similar to VIII, except filter paper treated with 5 ml. of concentrated HCl prior to ashing.

The data of table 2 show the following order for the various test solutions, given in their respective degree of effectiveness on the stimulation of dry matter production: (1) Autoclaving the filter paper with the NH_4-tartrate solution and then adding the other nutrients gave the most effective solution. This was fol-

lowed closely by a solution prepared by treating the nutrients with a HCl-ash of the filter paper. (2) Significantly lower in effectiveness was the solution which resulted by autoclaving the filter paper with the dextrose solution alone and then adding the other compounds. (3) Filter paper autoclaved in water, when supplemented with the other compounds, gave the next lower stimulative power. (4) The ash of filter paper, plus all the nutrients, ranks next. (5) Four substances when autoclaved separately with filter paper produce solutions which constitute the fifth class: $MgSO_4$, KH_2PO_4, all stock solutions, and ferric tartrate. (6) The control, which contained no filter paper or products thereof, was markedly lowest.

TABLE 2

METHODS IN PRODUCTION OF SYNTHETIC SUBSTANCE B

CULTURE	AVERAGE DRY WEIGHT OF RHIZOPUS MYCELIUM (MG.)	F±SE	SPORULATION
Control	79.8	1.00	++
I	98.3	1.23±0.009	+++
II	104.2	1.31±0.008	+++
III	113.4	1.42±0.018	+++
IV	100.8	1.26±0.010	+++
V	100.8	1.25±0.008	+++
VI	107.1	1.34±0.017	+++
VII	97.6	1.22±0.008	+++
VIII	102.9	1.29±0.011	+++
IX	110.5	1.39±0.018	+++

Since autoclaving the filter paper with either NH_4-tartrate or dextrose resulted in a greater stimulating value than when filter paper was autoclaved with water alone, it might be inferred that the co-factor enters into an organic salt combination chemically to form a growth factor for the *Rhizopus*. No explanation is offered to account for the lower value obtained when all the stock solutions were autoclaved with the filter paper. Since the ash, and especially the HCl ash, will substitute for the filter paper itself, it is most probable that this co-factor is inorganic in nature.

III. FURTHER METHODS IN PRODUCTION OF SYNTHETIC SUBSTANCE B

It was realized from the preceding that the reautoclaving, necessary for sterilization, introduced a factor which precluded definite proof that this co-factor did combine with one of the organic compounds and not with one or more of the compounds used. It was deemed advisable, therefore, to prepare and to test the following solutions:

C. Control.

I. Filter paper autoclaved with all stock solutions, then removed by filtration and filtrate sterilized and tested.

II. Similar to I, except that the Reeve Angel filter paper was replaced by an "ash-free" paper, Whatman no. 42.

III. Filter paper and NH_4-tartrate solution autoclaved and paper then removed by filtration. Filtrate sterilized by autoclaving. Remaining stock solutions mixed in proportions specified and heat sterilized, then mixed and tested.

IV. HCl-ash suspended in water for 30 minutes, filtered, and filtrate retained. Stock solutions added to filtrate and mixture heat sterilized. Resultant solution tested.

V. HCl-ash suspended in water for 30 minutes, filtered, and filtrate sterilized by autoclaving. Stock solutions mixed and heat sterilized, then mixed and resulting mixture tested.

TABLE 3

METHODS IN PRODUCTION OF SYNTHETIC SUBSTANCE B

CULTURE	AVERAGE DRY WEIGHT OF RHIZOPUS MYCELIUM (MG.)	F±SE	SPORULATION
C	84.1	1.00	++
I	107.8	1.28±0.015	++
II	98.6	1.17±0.013	+++
III	86.9	1.03±0.019	++
IV	102.6	1.22±0.022	+++
V	73.4	0.87±0.015	+++

The data of table 3 indicate that the slight augmentation in yield for the culture growth on test solution III, as compared with the control, is without significance. This fact tends to prove that the autoclaving of filter paper with the NH_4-tartrate solution does not result in a chemical formation of substance B. However, it does result in the growth factor formation when both the nitrogen- and carbon-nutrient sources are present, as shown by the results with the first two solutions. A tentative explanation for the discrepancy between the stimulative powers of the NH_4-tartrate treated solutions in the two tests is as follows, for the value of 1.42 for culture III in table 2 and for the value of 1.03 for culture III in table 3. In the former test, the second autoclaving permitted a reaction, at high temperatures, between the soluble substances of the filter paper and all the compounds used. In the latter test, a reaction of the NH_4-salt and the paper alone was possible at the higher temperatures. A comparison of these two results would lead to the inference that the autoclaving of NH_4-tartrate and filter paper does not result in a significant production of synthetic substance B.

The acceleration of growth resulting from the use of the "ash-free" paper, Whatman no. 42, as compared with the other, indicates the possibility that perhaps the co-factor is not metallic. This ash-free paper does contain, however, traces of metallic ions and leaves a small but perceptible ash, when treated in a muffle furnace. A comparison of the results obtained with test solutions IV and V gives an interesting picture of the possible mechanism involved. The metallic ions, obtained by ashing filter paper, are toxic in themselves but do result in the formation of a marked stimulator when autoclaved with the stock solutions.

Tentative conclusions

1. Commercial filter paper contains a water-soluble co-factor for *Aspergillus niger* and *Rhizopus suinus*.

2. This so-called co-factor is possibly metallic in nature and tends to be toxic to the vegetative growth of *R. suinus*, when used singly.

3. The co-factor enters into the synthetic formation of a growth factor, of the substance B group, when autoclaved with an organic ammonium salt and a sugar.

4. This chemically formed substance B may be an organic salt of some trace element, which is in accord with the suspected nature of the biologically produced substance B.

Sincerest thanks are hereby extended to Dr. B. M. DUGGAR, under whom this work was conducted and the protocol of this paper prepared.

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EFFECT OF VITAMINS ON GROWTH OF RADISH AND CAULIFLOWER[1]

E. C. MINNUM

It has been stated that the use of vitamins B_1 and B_2 added in solution to intact plants grown in sand or gravel with added nutrients results in increased growth (**1, 2**). Others have not been able to obtain these effects (**3, 4**), either in sand culture or in ordinary garden soil. In most of these experiments the concentration of B_1 used was 0.01 mg. per liter of solution, with a few reporting quantities up to 1 mg. per liter. This paper reports the effects of higher concentrations.

Experiment with radish

Eight-inch clay pots were painted on the inside with a special nontoxic asphalt paint and filled with a no. 1, fine quartz sand. The hole in the bottom of each pot was plugged with glass wool. All pots were leached three times with distilled water previous to planting the seeds.

Approximately twenty untreated seeds of Scarlet Globe radish were planted in each pot to a uniform depth, and these were later thinned to an even stand of eight plants per pot. Each treatment consisted of five replications, so that there was a total of forty plants per treatment. The pots were randomized on the greenhouse bench by drawing numbers out of a box.

The experiment was set up in the 50°–60° F. greenhouse on November 28, 1940, at Ithaca, New York. The mean daily temperature was close to 57° F. No additional light was given, the plants receiving only that normal for the period. With the exception of a few sunny days the weather was mostly overcast during the growing period, there being 27 per cent of possible sunshine in December and 27 per cent in the following month up to January 22.

Hoagland's nutrient solution plus minor elements were added at intervals of 3 or 4 days in amounts sufficient to leach through the pot. Distilled water was added on a few occasions, usually after a sunny day. All pots were thoroughly leached with distilled water once a week.

Pure crystalline vitamins B_1, B_2, and B_6 were used. Fresh solutions were made up each time the pots were treated by weighing out the necessary number of milligrams of the vitamin and adding a sufficient amount of distilled water so that 1 cc. of the solution contained 1 mg. of the vitamin. From these stock solutions dilu-

[1] This investigation was financed largely by funds provided by the Grange League Federation, Ithaca, New York, and Nopco Laboratories, Harrison, New Jersey. Paper no. 238, Department of Vegetable Crops, Cornell University, Ithaca, New York.

tions were made for those treatments requiring smaller amounts of the vitamins. Vita Flor, a commercial compound containing 0.1 per cent of vitamin B_1, 0.5 per cent nicotinic acid, and traces of vitamins B_2, B_6, and pantothenic acid, was also included.

The vitamins were added to sufficient water to make 300 cc. of solution per pot per treatment, while the checks were given 300 cc. of distilled water. Additions of the vitamins were generally made on the morning following application of the nutrient solution. Vitamins were applied on December 5, 9, 14, 17, 20, and 23, 1940, and January 1, 4, 8, 12, and 15, 1941. The radishes were harvested on January 22.

Growth of the plants was steady, considering the amount of cloudy weather prevalent during the short days of winter. A slight light green area was noticed around the margin of the leaves of many of the plants. This cannot be attributed to the treatment as it was present also on the adjacent greenhouse bench where the same seed was used in a fertilizer experiment in soil with different levels of N, P, and K. No one fertility level had more leaves with light green margins than another, so apparently this was a genetic factor rather than nutritional.

To check the effect of the vitamin applications on the pH of the sand, composite samples were taken several hours after treatment from those pots receiving the highest applications of vitamins. The determinations were made by the quinhydrone method, and the pH was found to average 5.45–5.90, with the checks at 5.73.

When the plants were harvested on January 22, the majority of the roots were of ordinary market size. The eight plants were pulled from each pot, the roots carefully shaken in water to remove adhering sand and then dried with paper toweling. Weights were made on a balance sensitive to 0.01 gm.

Table 1 shows the number of milligrams of vitamins applied per pot per treatment. The figures for the fresh and dry weights of the plants are the means for each treatment, consisting of five pots of eight plants each. While some individual treatments resulted in significant increases, none was significantly greater or less than the checks. No treatment, even those where 10 mg. of the vitamin was applied per pot per treatment, had a significantly depressing effect on growth. Apparently 10 mg. of vitamins per pot was entirely without effect, either beneficial or detrimental.

Experiment with cauliflower

Cauliflower seeds of the variety Super Snowball were planted on November 24, 1940, in flats of pure, washed, fine quartz sand. On December 11 they were transplanted to 5-inch new clay pots that had been previously painted on the inside with the nontoxic asphalt paint.

Ninety-six pots were placed in both the 60°–70° and 70°–80° F. greenhouses.

The ninety-six pots in each house were divided, one-half being put on long day from January 11, 1941, to harvest on February 6, 1941, by using four 100-watt bulbs suspended approximately 18 inches above the surface of the leaves from 5:00 to 11:00 P.M. Black cloth curtains hung on the four sides at 5:00 P.M. each day prevented the light from affecting adjacent plants.

Twelve pots at each temperature from both the long- and short-day treatments were reserved as checks. Twelve were treated with 0.01 mg. of vitamin B_1, twelve with 0.1 mg., and twelve with 0.5 mg. on the following dates: December 14, 19, 23, and 28, 1940, and January 3, 7, 11, 15, 20, and 25, 1941.

TABLE 1

Treatment	Fresh weight (gm.)			Dry weight (gm.)*	
	Whole plants	Roots	Tops	Roots	Tops
0.01 B_1	49.58	27.46	22.10	1.45	2.00
0.5 B_1	48.42	27.90	20.52	1.54	1.81
1.0 B_1	40.14	19.90	20.24	1.26	1.92
5.0 B_1	49.48	27.04	22.24	1.48	1.98
10.0 B_1	48.86	26.02	22.84	1.21	2.13
0.01 B_2	47.50	25.64	21.86	1.44	1.85
0.5 B_2	48.72	24.18	24.54	1.36	2.34
1.0 B_2	50.26	25.66	24.60	1.40	2.13
5.0 B_2	47.12	25.36	21.76	1.17	1.95
10.0 B_2	38.66	18.16	20.50	1.11	1.93
0.01 B_6	46.18	24.04	22.14	1.58	2.07
0.5 B_6	45.34	24.80	20.36	1.41	1.98
1.0 B_6	44.56	23.64	20.92	1.29	1.99
5.0 B_6	44.56	22.06	22.50	1.28	2.18
10.0 B_6	42.38	22.08	20.30	1.40	1.77
Vita Flor 1 cc.	40.22	19.98	20.24	1.17	1.97
Vita Flor 2 cc.	39.52	21.10	18.42	1.18	1.96
Vita Flor 5 cc.	36.02	17.26	18.76	0.98	1.98
0.01 B_1, B_2, and B_6	44.22	23.58	20.64	1.27	1.96
0.1 B_1, B_2, and B_6	47.90	27.58	20.32	1.50	1.99
Check	43.60	25.68	17.92	1.36	1.70

* Differences not significant. Difference of 11.93 necessary for significance at odds of 19:1.

Hoagland's complete nutrient solution plus minor elements were added to the pots approximately every fourth day, and when necessary distilled water was added between the nutrient applications. Freshly made vitamin solutions were used, dilutions being made from a stock solution containing 1 mg. of the pure crystalline vitamin per cubic centimeter of solution. When the vitamins were applied, they were so diluted that each plant received 10 cc. of distilled water with the vitamin included. It was felt, especially during the early stages of growth when the plants were small, that if any effect was to be obtained, 10 cc. of the solution would be more effective than the same amount of vitamin in a larger amount of water.

Growth was steady in both houses, and a few days after the lights were used increased growth was evident on those plants receiving the extra light.

All plants were harvested on February 6. Weights were taken on a balance sensitive to 0.01 gm. and the data subjected to analysis of variance. In neither house was there any significant difference between the treatments and their checks.

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NUMBER OF CHLOROPHYLL COMPONENTS

F. P. ZSCHEILE

In 1934 the writer presented certain evidence (**8**) which indicated that normal green leaves might contain a third component, *c*, in the chlorophyll complex, in addition to components *a* and *b* which were originally found by WILLSTÄTTER and STOLL (**6**). The evidence for this additional component was incomplete and inconclusive. BAKKER (**1**), WINTERSTEIN and SCHÖN (**7**), and MACKINNEY (**4**, **5**) presented evidence unfavorable to ZSCHEILE's suggestion of a third component. In the meantime, after the development of more refined methods for chlorophyll preparation and the availability of better absorption data on the products formed by treatment of chlorophyll with acid, the writer has re-investigated this problem and considers that a note is desirable to clarify the subject.

In 1936, after the criticisms of WINTERSTEIN and SCHÖN (**7**) concerning the use of talc were at hand, components *a* and *b*[1] were prepared with sucrose substituted for talc, other conditions remaining much the same as described in 1934 (**8**). All solutions were kept at 5° C. except while being studied. Sucrose was washed from the ethereal chlorophyll eluates with water. To force the solution through the adsorbent column, a pressure of 5 lb. of nitrogen was used instead of suction. The use of glass columns approximately 10 inches long permitted their more exhaustive washing.

The zones formed were very similar in color to those reported by ZSCHEILE with talc. Some brown pigment separated out at the top of the column and was discarded. The characteristic "*c*" fraction, green in color, was isolated by adsorption on a second sucrose column (5 inches in length). It separated completely from the 5-mm. zone of brown pigment, which remained at the top of this column. No blue layer of *a* appeared at the bottom. The *a* fraction, after adsorption on a second column, produced no brown layer whatever and was uniformly blue-green on the column, yielding a blue ether solution. The *b* fraction from extracts 11–18 (**8**) was dissolved in 30 per cent ether—70 per cent petroleum ether and chromatographed upon a sucrose column. No blue layer of *a* appeared until the column was developed with 40 per cent ether plus 60 per cent petroleum ether. Then a narrow 5-mm. band of blue *a* separated completely from the green at the bottom of the column. The lower 15 mm. of the green zone was lighter in color than the main body (140 mm.) and was discarded because of possible contamination with the *c* fraction. A thin brownish region at the top was also discarded. All samples were

[1] These preparations were made in the department of chemistry, University of Chicago.

washed four times with water while dissolved in ether. Most of the solvent was then evaporated under reduced pressure, and the sample was dried *in vacuo* at a pressure of 2×10^{-5} mm. of mercury, as measured on a Macleod gauge.

Absorption measurements were then made on ether solutions with the photoelectric spectrophotometric apparatus described by HOGNESS, ZSCHEILE, and SIDWELL (**3**). A mixture of ether solutions of these component preparations (*a* and *b*) containing 48 per cent *b* by weight was studied. Its visible absorption curve was intermediate between the curves for the single components, and the curve for the mixture agreed closely with the others at their crossing points, indicating that the components of a mixture may be identified from its absorption curve if such curves for the components are known. The *a* fraction was washed four additional times with water, to remove any possibility of sucrose contamination, and dried. Absorption values at the principal maxima were lowered 4 per cent by this treatment.

A few drops of 22 per cent HCl were added to ether solutions of *a* and *b* to cause pheophytin formation. Curves of these products of acid treatment resembled those recently published by ZSCHEILE and COMAR (**9**).

The *c* fraction, which was apparently separated completely from *a* and from brown decomposition products on the sucrose column, was next considered. Its absorption curve was similar to those of earlier preparations from talc columns, so far as positions of maxima were concerned. It was noted that the maxima in the blue and red regions corresponded in wave length exactly to those of both chlorophyll *b* and "pheophytin *a*." Moreover, when the absorption curve for the *c* fraction was raised by multiplication of the absorption coefficients by the necessary factor 1.27 (presumably due to colorless impurities) to make it cross the other curves at one of their crossing points, 4210A (4235A according to **9**), it was found that all three curves agreed closely at all the crossing points for chlorophyll *b* and pheophytin *a*. There were five such points in the visible spectrum. The shape of the curve for the *c* fraction indicated that this fraction consisted chiefly of chlorophyll *b* and pheophytin *a*, with the latter predominating. All the principal bands for these two compounds were present as maxima, and those for pheophytin *a* were more intense than those for chlorophyll *b*. Other combinations of chlorophyll and "pheophytin" pigments are eliminated by these considerations. The absorption spectra thus demonstrated that a homogeneous zone on an adsorption column may, under certain conditions, contain two adsorbed compounds.

Recent work in these laboratories has indicated several sources of error in the results of 1934 (**8**). Some of these were recently discussed by ZSCHEILE and COMAR (**9**), whose experiments have indicated the probable reasons for ZSCHEILE's 1934 results. The fact that all chlorophyll preparations from the writer's laboratory were dried previous to 1939 makes further detailed comparison of results futile.

It is concluded that the earlier observations on component *c* were the result of the decomposition of chlorophyll *a* to pheophytin *a* and of the fact that the chromatographic methods employed were inadequate to separate them. After consideration of more recent results (**9, 2**) it would seem that both the use of talc and the long time of preparation required in the earlier method were factors which could have caused the formation of pheophytin *a*. The advantage and usefulness of the spectrophotometric method were demonstrated as means of control during chromatographic separations.

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CURRENT LITERATURE

A Revision of Melanconis, Pseudovalsa, Prosthecium, and Titania. By LEWIS E. WEHMEYER. Ann Arbor: University of Michigan Press, 1941. Pp. 161. Pls. 11. $2.50.

This is an important contribution to our knowledge of a difficult group of the Pyrenomycetes. The introduction sketches the history of the genera treated and discusses the criteria used in segregating the different groups. The author realizes that there are in most cases no fixed lines of demarkation between genera and species. The current system of classification, based chiefly on spore color and septation, is artificial and leads to confusion of natural groups. Natural relationships can be determined only by study of the complete life cycles and microscopic characters of the plants. The author concludes that genera "are almost impossible to define," and "A genus, therefore, is generally an artificial conception, has arbitrary boundaries and should be used in a manner to serve most efficiently the two purposes mentioned—as an indication of relationships and as a convenient index tab."

An attempt to attain this laudable purpose could hardly be expected to result in a perfect agreement with other students of the group. The author's generic concepts are commendably conservative. New genera and species, frequently numerous in such revisions, are noticeably lacking; such new names as are used are mostly applied to subgeneric and sectional groups. The adoption of *Prosthecium* for species found heretofore mostly in *Aglaospora* and *Pseudovalsa* results in a number of new combinations. The chief difference between *Prosthecium* and *Pseudovalsa* is the presence of a hyaline appendage on each end of the ascospores. Whether this character is of generic value is a matter of opinion. The demonstration of such appendages depends in many cases upon the age and condition of the specimen. The separation of *Massaria* from *Aglaospora* on the 4-spored asci of the latter genus is properly recognized as doubtful. Cultural and cytological studies are needed to clear up this point.

The author has had unusual opportunity to study type and authentic specimens of most of the older species in this group of fungi and is thus able to give very complete synonymy. It is unfortunate that the specimens in the mycological collections of the Bureau of Plant Industry were not examined, as they would have given additional information regarding the distribution of the species in this country and perhaps the identity of some of the still doubtful ones. Rather too serious attention seems to have been given to the frequently modified and still unstable rules of nomenclature. This has led to the rejection of some of our old and generally used specific names. For example, the common species *Aglaospora profusa* (Fr.), published on p. 392, Fr. Syst. Myc. 2, has been replaced by *A. anomia* (Fr.), published on p. 381 of the same work. It is to be hoped that some day, in the interests of uniformity and stability, mycologists will abandon this procedure in regard to specific names, as they are already doing with generic names, and conserve the well-known ones. Such changes lead only to confusion and are a detriment to the advancement of systematic mycology, and it would seem preferable to delay making them for the present, as some of our British colleagues are doing, hoping that the next Botanical Congress will approve the conservation of specific names.

This excellent monograph adds extensively to our knowledge of the older species and their synonymy and also of their conidial stages, which have in many cases been proved by pure

cultures. It is a contribution of such importance and value that criticisms of it seem trivial.—C. L. SHEAR.

Die Methoden der Fermentforschung. Edited by EUGENE BAMANN and KARL MYRBÄCK. Leipzig: Georg Thieme, 1940–1941.

This compendium of methods of enzyme research began to appear early in 1940, and to date eight Lieferungen have appeared, totaling 3047 pages. Each section of the monograph has been prepared by some worker familiar with the special methods employed in some restricted field of enzyme chemistry. The introductory section considers the nature of enzymes, the mechanism of enzyme reactions, a general discussion of methods of enzyme research, and the nomenclature and systematic classification of enzymes.

The following special sections are three in number. The first deals with the substrates used in enzyme research, their preparation, properties, and applicable investigational techniques; the intermediate products formed during reactions; and the end products. This section also discusses the more recent research methods used in substrate investigations and the general methods of research on enzymes, such as methods of following catalysis, obtaining and testing enzyme preparations, isolation and characteristics of lyo- and desmo-enzymes, general procedures in concentration and separation of enzymes, and the determination of the general properties of enzymes.

The second division takes up special groups of enzymes, such as the hydrolases, the desmolases, and enzymes of biological oxidation and reduction, assimilation, antienzymes, and enzyme models.

The third section is devoted to technical research on enzymes in industry, such as fermentation of grains, etc., processing of fats, manufacture of malt extracts, and the baking industries. The last part of Lieferung 8 considers research methods used in clinical problems.

This excellent compilation should be in the hands of, or accessible to, all students of enzyme reactions. Naturally it is impossible for such a compendium to attain completeness or perfection, but a work which draws together thousands of papers which have appeared in periodic literature has exceptional value. About 130 individual scientists have co-operated in this effort, and approximately 200 separate reports are included.

It is remarkable that in a world so torn with strife, scientific men still go forward serving the arts of peace. This fine example of co-operation is worthy of the highest commendation. The total price of the eight Lieferungen issued is RM 233.7, or somewhat less than $100. Because of the great expense of publication, the printer cannot offer them except in complete sets.—C. A. SHULL.

The Cytoplasm of the Plant Cell. By ALEXANDRE GUILLIERMOND. Translated by LENETTE ROGERS ATKINSON. Waltham, Mass.: Chronica Botanica Co.; New York City: G. E. Stechert and Co., 1941. Pp. 247. $4.75.

This volume, which deals with the cytoplasm and its inclusions, is characterized by the thoroughness with which it has been done, the clarity of presentation, and the fairness with which the older and newer views in cytology are brought together and integrated.

There is a succinct and informative foreword by SEIFRIZ, following which there are twenty chapters. The first presents a historical sketch of the general subject, points out the difficulties in the study of cytoplasm, and recommends methods. The second is devoted to general facts on the structure of the plant cell, its cytoplasm and morphological constituents, while the follow-

ing three chapters give detailed attention to the physical properties and general characteristics, chemical constituents, and physico-chemical constitution of the cytoplasm. Six chapters are next concerned with plastids and the chondriome, the relationship between chondriosomes and plastids, the duality of the chondriome, and hypotheses relative to the roles of chondriosomes and plastids. Vacuoles, including methods of vital staining, development, origin, and significance, and the role of the vacuolar system and hypotheses concerning it, are discussed in the succeeding five chapters. Golgi apparatus, canaliculi of Holmgren and other cytoplasmic formations, lipside granules, microsomes and other metabolic products, and cytoplasmic alterations occupy the next three chapters. A general summary concerning the present knowledge of the cytoplasm, the chondriome, plastids, and the vascular system or vacuome is given in the final chapter.

The present volume is the first addition, printed in America, to the list of books which Dr. Frans Verdoorn is editing and publishing under the title, A New Series of Plant Science Books. MRS. ATKINSON has accomplished an excellent job of translating and interpreting for English readers a highly stimulating work, which can be recommended to botanists generally.—J. M. BEAL.

Native Midwestern Pastures; Their Origin, Composition and Degeneration. By J. E. WEAVER and W. W. HANSEN. University of Nebraska Conservation and Survey Division: Nebraska Conservation Bulletin no. 22, 1941.

Regeneration of Native Midwestern Pastures under Protection. By J. E. WEAVER and W. W. HANSEN. University of Nebraska Conservation and Survey Division: Nebraska Conservation Bulletin no. 23, 1941.

These two papers represent another step forward in the careful studies by which WEAVER and his students have made the central portion of fragmented true prairie the best known of all native grasslands. In the first, five stages in the degeneration of native prairie under the influence of grazing by domestic stock are set forth, together with the various types of vegetation resulting. This task, based originally on studies begun in 1929, was made more difficult by the effects of the droughts of 1934–40 in accelerating degeneration or in causing the almost immediate destruction of some types. The earlier survey permitted an evaluation of the responses of the various species to grazing alone; and the conclusions, confirmed by clipping experiments, are embodied in lists and discussions of prairie grasses and forbs that decrease under grazing, those that increase, and grasses and forbs that invade pastures. The changes in pasture types that have resulted from drought are clearly set forth. The second bulletin deals with the changes in the plant population over a 4-year period, 1937–40, which resulted from the complete protection from grazing of a pasture near Lincoln, classed in 1932–33 in the third stage of degeneration. The recovery of the prairie grasses, based on stem counts in permanent quadrats, is excellently depicted in graphs.

This study also includes a comparison of the yields, in 1940, of pasture under protection for 1, 2, and 4 years, and prairie. Based on clipping at various intervals, yields were 1.15, 1.51, 1.31, and 1.12 tons per acre, respectively. When evaluated on a probable utilization basis, the comparative figures are 0.49, 0.72, 0.74, and 0.76 tons. Based on the better forage grasses, one year of complete protection more than doubled the production of usable material, and 3 years of protection increased it eight times.—C. E. OLMSTED.

Genetics and the Origin of Species. 2d ed. revised. By THEODOSIUS DOBZHANSKY. New York: Columbia University Press, 1941. Pp. xviii+446. $4.25.

Although only 4 years have elapsed since the first edition of this highly stimulating book appeared, such progress has occurred in certain areas, notably in population genetics and cytogenetics, that not only substantial additions but complete rewriting of certain portions of the book were found necessary. The organization and the chapter headings of the former edition have been retained, and the book has been extended by some 82 pages.

Again the author has succeeded in bringing into proper relation to one another the accumulation of information on mutations of all sorts (genic and chromosomal), on genetic composition of populations, on species differences, and on the mechanism of hybrid sterility.

The volume comprises an extraordinary amount of factual material, but this is so thoroughly organized and integrated as to make the book highly readable throughout. As in the former edition, one of the most valuable features is the incorporation of the work of European—and especially of Russian—authors, whose results have often been overlooked in America. It is a necessary book for all who are interested in recent developments of the theory of evolution.—J. M. BEAL.

Phylogenetic and Cytological Studies in the Ranunculaceae. By WALTON C. GREGORY. Trans. Amer. Phil. Soc., New Series, Vol. XXXI, Part V. 1941.

A study of the phylogeny and systematics of the Ranunculaceae, chiefly on the basis of the type and number of chromosomes. In all, the chromosomes of 109 species involving 19 genera have been studied, with 43 species from 8 genera being reported for the first time. The following basic chromosome numbers have been selected for the family: 5, 7, 8, 9, and 13. The highest degree of polyploidy has been reached in the 7- and 8-series, with the highest $2n$ numbers-154 and 128, respectively.

An evaluation of the significance of polyploidy and of the relative roles of polyploidy as against other factors in evolution of the woody habit in the Ranunculaceae has been attempted. A phyletic rearrangement of the genera and tribes of the family has been made on the basis of chromosome type, size, and basic number. On the same data a polyphyletic origin of the various tribes of the family from some ancestral group has been suggested and illustrated by phylogenetic charts, comparing the classical and the author's classifications.—J. M. BEAL.

Cytology, Genetics, and Evolution. By M. DEMEREC *et al.* Philadelphia: University of Pennsylvania Press, 1941. Pp. v+168. $2.00.

Contains twelve addresses presented at the University of Pennsylvania's Bicentennial Conference, September, 1940: The nature of the gene, by M. DEMEREC; Chromosome structure, by CHARLES W. METZ; The sex chromosomes: heteropycnosis and its bearing on some general questions of chromosome behavior; chromosomal interchanges, by ALBERT F. BLAKESLEE; Chromosomal differences between races and species of *Drosophila*, by TH. DOBZHANSKY; Evolution of the germplasm, by CLARENCE E. MCCLUNG; Hereditary status of the rhizopods, by HERBERT S. JENNINGS; Nuclear behavior and reproduction in ciliated protozoa, by WILLIAM F. DILLER; Inheritance in ciliated protozoa, by T. M. SONNEBORN; The physico-chemical properties of the nucleus, by LEON CHURNEY; The chromosomes of the amphibian nucleus, by WILLIAM R. DURYEE; Radiation and the cell nucleus, by PAUL S. HENSHAW.—J. M. BEAL.

Tabular Keys for the Identification of the Woody Plants. Compiled and arranged by FLORENCE B. ROBINSON. Champaign, Ill.: Garrard Press, 1941. Pp. iv+156. Spirally-bound, loose-leaf, planographed. $2.50.

These keys are intended for field use and for quick reference to 500 trees, shrubs, vines, conifers, and broad-leaved evergreens growing in the northern states and Canada. There are separate groups of dichotomous keys to the genera for each of the groups of plants just named and for the leafy and the winter condition separately. The species are determined by referring to tabular keys of each genus, alphabetically arranged. Instruction in the use of these keys, a glossary, and nomenclatural lists of species, together with the common names, add to the usefulness of this work.—P. D. VOTH.

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